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DEVELOPMENT AND MECHANISM OF DISCHARGE IN SPHAEROBOLUS IOWENSIS N.SP. AND S. STELLATUS TODE

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Plates 16–25

A *Sphaerobolus*, with its interesting mechanism for glebal discharge, is one of the most spectacular and fascinating of fungi. Fischer (3) in 1884 reviewed previous work upon *Sphaerobolus stellatus* and published the first comprehensive study of its development. Four years later Rabinowitsch (10), one of Fischer's students, added more details especially as to the development of the so-called "collenchyma" layer. Pillay (9), another of Fischer's students, in a recent paper, published after my studies were nearly completed, gives the results of still more detailed studies on *S. stellatus*. In each of the above papers a single strain was considered. The writer has studied four strains of *Sphaerobolus*, three American and one European. Since these four strains show considerable variations from each other and from the accounts mentioned above, a further discussion of these forms is needed.

Materials

No authentic specimens of any species of *Sphaerobolus* have been available for comparison and all descriptions are so inadequate that positive determinations are difficult.[1]

[1] Specimens of the three American forms were sent to Dr. Roland Thaxter of Harvard University. These were turned over by him to Dr. C. W. Dodge for comparison with specimens in the Gray herbarium. Dr. Dodge writes: "Most

The four strains of *Sphaerobolus*, which for convenience are designated as SI, SII, SIII, and SIV, have been maintained in cultures and an abundance of material was available for study and comparison. Each of the four strains will be discussed below:

SI. The specimens from which my cultures of SI were obtained were found November 23, 1916, in a local greenhouse, growing on particles of organic matter in the soil of a carnation bench. There were only a few fruit-bodies and from these cultures were made. The specimens were placed in a sterile Petri dish and allowed to discharge their glebal masses which adhered to the walls of the dish. These were removed and planted upon sterile agar plates. A pure growth of white mycelium, with abundant clamp connections, resulted in each case. This mycelium was transferred to flasks containing sterile agar-agar media, corn-meal mush, and partially rotted willow wood. The cultures were placed in the light. Cultures upon willow wood gave an abundance of fruit-bodies within a few weeks but those upon corn-meal and agar media never fruited. Fruiting cultures are shown on plate 16. Figure 1 shows a young culture on willow wood in a liter flask. This culture is just beginning to fruit. Figures 3 a and b are from older willow wood cultures. Cultures have since been maintained constantly and various media tried. The best results have been obtained upon willow wood, half rotted horse dung, sawdust (probably willow or ash), and mixtures of horse dung and sawdust. Figure 4 shows a part of a culture on sawdust. SI, as will be seen later, agrees so closely with the accounts of other workers, cited above, that it seems evident it is the same as their *S. stellatus*. It also seems to be the same as a *Sphaerobolus* collected several times by the writer during the summer of 1917 in the vicinity of Ithaca, N. Y. No cultures were made from these collections hence no definite comparisons can be made. Specimens in the University of Nebraska herbarium collected from an old board walk at Lincoln, Nebraska, are seemingly also the same as SI.

The second form of *Sphaerobolus* (SII) studied was collected early in September, 1917, by Dr. H. B. Brown at Starkville, Miss. This form had grown apparently on horse dung. It was sent by him to the late Prof. G. F. Atkinson of Cornell University. Professor Atkinson, know-

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of the *Sphaerobolus* species of which we have authentic material have been or ought to be excluded from the genus according to Farlow's mss. notes on envelopes. We have no authentic material of *S. stellatus*, to which nearly everything has been reduced by some one or another."

ing of my cultures of *Sphaerobolus* (SI), immediately forwarded it to me for comparison. Cultures of this fungus were obtained in the same way as those of SI and have been ever since similarly maintained. It has a much more vigorous mycelial development[2] and rarely fruits in cultures. When fruit-bodies develop they are about twice the size of those of SI.

The third type of *Sphaerobolus* (SIII) was found by the writer during the summer of 1919, growing on the coniferous boards of an old porch at Hunters, near Spirit Lake, Iowa. The basidiocarps appeared to be more sunken in the substratum, did not open as widely as other specimens previously observed and the glebal mass was darker in color. Because of these variations in appearance cultures were made in September from materials brought to Lincoln. The same methods were used as with the preceding types. This form was again abundant upon the same porch during the summer of 1920 but was not found at any time in any other place in the vicinity. This form has so many distinctive characteristics, as will later be seen, that it must be considered as specifically distinct. Figure 5[3] shows a few basidiocarps developed in a culture.

Publication of work upon these three forms was delayed in order that these three American forms might be compared with at least one European form of *Sphaerobolus*. This fourth strain (SIV) was obtained from the Central-Bureau voor Shimmel Culture, Barn, Holland, and had been isolated by Kniep who called it *S. carpobolus* (= *S. stellatus* Tode). This culture has been maintained since the summer of 1924 and is undoubtedly the same species as SI for all minor variations occur.

Materials for developmental studies were taken from young, actively growing cultures and fixed in various solutions. Materials aspirated and then fixed in Fleming's strong solution gave the best results but none of the fixing reagents used gave really satisfactory fixation, especially in the glebal region of older basidiocarps. The poor fixation secured in this region seems very definitely to be due to the development of an oily slime which prevents the penetration of the fixing reagents. That Fleming's solution did not penetrate is evident from the fact that although much oil is present in the gleba, as shown by tests with Sudan III, little or no blackening occurred except in a thin peripheral layer.

[2] If glebal masses of the three forms (SI-SII-SIII) were planted under the same condition on agar plates by the time the mycelium of SI spread an average of 8 mm. that of SII had spread 16 mm. and SIII, 5 mm.

[3] Unless otherwise stated the figure references in this article are to the figures on plates 16–21. Figures on plates 22–26 are referred to by plate number also.

The oil is soluble in absolute alcohol but alcoholic fixing solutions tried have not given better results.

FORM AND STRUCTURE OF NEARLY MATURE BASIDIOCARPS

The basidiocarps of the four strains of *Sphaerobolus* look very similar. Just previous to opening they are spherical in shape and more or less deeply imbedded in the surrounding mycelium and substratum (see rounded masses, figs. 3 and 4). Their color is a light yellow as the deep yellow of the interior shows faintly through the fine white filaments covering the exterior. When mature the peridial layers of the basidiocarps break open on top in a stellate manner (figs. 1–5) revealing a spherical glebal mass on the inside. This rupture takes place during the early morning. As the morning advances a clear watery substance[4] accumulates around the glebal mass in the cup formed by the peridial parts (fig. 2). Later in the morning when the peridial cup is well filled a rupture takes place between two regions of the peridium (pl. 22, above), the inner of which becomes inverted above the outer in the form of a glistening dome, and the glebal mass is forcibly ejected to a height of several meters, as will be later shown. The black specks on the culture flask in figure 1 are discharged glebal masses. Figures 3a and 3b are two views of the same culture taken about a half hour apart and show the changes spoken of above, three of the four basidiocarps to the left having discharged in the meantime. The inner peridial layer may be forcibly ejected also. This is usually the case in SIII and may also occur in the other forms.

Three very definite parts may be distinguished: First, an outer peridial part which always remains attached to and more or less deeply imbedded in the substratum; second, an inner peridial part which at the time of discharge becomes inverted as a dome above the outer part and which by its sudden change of position hurls the glebal mass; and third, the glebal mass itself.

Sections of basidiocarps nearing maturity show the structure of these three regions which furnish the mechanical basis for the discharge. Figures 44, 45, and 49 show median longitudinal sections of nearly mature to mature basidiocarps of SI, and figures 47 and 50 show higher

[4] This watery substance can be easily drawn up in a capillary pipette. It contains a great deal of maltose as indicated by Flückiger's reaction and by osazone formation.

magnifications of the outer portions at the apex and base of a basidiocarp. Similarly figures 15, 16, 21, and 22 show the structures as seen in SII and figures 35, 41, and 42 the corresponding structures for SIV. As will be noted in the figures mentioned above the outer peridial region of SIII differs structurally from that of the other three forms. The details of this region as occurring in SI, SII, and SIV are shown on plate 22, figure 1, as well as in figures 41, 42, 47, and 50. It consists of an outer layer of loosely interwoven mycelial hyphae designated as "a," a gelatinous layer with very loosely interwoven hyphae "b," and an inner pseudoparenchymatic layer "c." The middle gelatinous layer "b" in the outer peridium gradually disappears as the basidiocarp matures (figs. 34, 35, 44, 49) and is entirely wanting by the time the discharge takes place (fig. 48). In SIII this middle gelatinous layer, so conspicuous in SI, SII, and SIV, is not found at any time, so in this form the outer peridial region consists only of the outer layer of mycelial hyphae and a pseudoparenchymatic layer. See figures on plate 17, plate 24, figure 4, and figures 21 and 22.

The inner peridium of all four forms consists of a layer of interwoven generally tangential hyphae ("d" in all figures cited above) and a palisade-like layer, the collenchyma layer of Fischer (3), Pillay (9), etc., where the cells are very much elongated radially at the base ("e" on pl. 24, fig. 4; pl. 22, fig. 1, right; and figs. 22, 41, and 50) and gradually become shorter on the sides and merge into a pseudoparenchymatic layer at the apex ("e" in figs. 21, 42, 47, and pl. 22, fig. 1, left). The tangential nature of the hyphae in the outer portion of the inner peridium can be seen best in a tangential section such as is shown in figure 40. It gives the appearance of a ball tightly wound with twine.

The glebal mass is surrounded by a pseudoparenchymatic layer ("f" in all figures mentioned above) and on the interior consists largely of spores and hyphae as is seen at "g" of the same figures. Just to the inside of the pseudoparenchyma surrounding the gleba, mingled with the filaments, may be found most abundantly the enlarged, densely filled cells known as the gemmae. A small portion from regions "f" and "g" is shown much enlarged on plate 22, figure 2. The spores are heavily stippled and the gemmae lightly stippled.

At maturity the hyphae in the glebal region break down almost completely giving rise to an oily slime in which the spores are firmly held.

CHANGES TAKING PLACE DURING OPENING OF BASIDIOCARPS AND DISCHARGE OF GLEBAL MASS

Where ruptures take place at maturity may perhaps be best seen in figure 12, a rare case in which a glebal mass was retained in an opened fruit-body. Usually the glebal mass was discharged from an open fruit-body as soon as it touched the fixing fluid. The position of the peridial parts after the discharge is shown in figure 48.

Although no exact data can be given for the conditions essential to secure vigorous discharges the following have been observed: First, an abundance of moisture in the substratum and a relatively humid atmosphere are absolutely necessary; second, light, preferably an east exposure; and third, a sudden rise in temperature to about 90°F. (30°C.) during the morning and lower temperatures during the remainder of the day. So closely associated are the effects of light and heat that it is difficult to determine the exact influence of each separately as exposure to the sunlight always causes a rise in temperature.

Cultures placed in south or west exposures never fruited abundantly. Cultures rarely formed basidiocarps during continued hot weather and formed relatively few during the winter months. The maximum fructification always occurred during April and May, each spring, and during the fall when nights were cool and days bright and warm. SII fruits at higher temperatures than the others.

Various experiments were conducted to determine how the structures function in bringing about the discharge and the source of the energy causing the discharge.

When a fruit-body first breaks open the glebal mass is firmly attached to the peridium at the base but can be removed with a needle. After the basidiocarp has been open an hour or more the glebal mass lies as a ball entirely free from the peridial walls and may be rolled over or removed with great ease. These glebal masses, which are slightly heavier than water, were removed at various periods after opening and tiny balls of paraffin mixed with sand to weight them to about the same specific gravity as the original mass were substituted. These were as readily discharged as the glebal masses. Pebbles of similar size were used as substitutes and also drops of mercury the size of or larger than the glebal masses. These were as readily discharged as the original projectile. During the time intervening between the substitution of the artificial ball for the glebal mass and the discharge, water accumulated in the peridial cup and all changes took place normally. Thus it was

clearly demonstrated that the glebal mass plays no part in producing the discharge.

That the outer peridial region takes no part in causing the discharge is quite evident from the following facts: First, that before the discharge takes place the inner and outer peridial parts separate from each other in the sinuses of the notches until a needle point may easily be inserted. This removes the possibility that gas formation between the peridial layers is in any way responsible for the discharge. Second, there are no morphological structures suggestive of such a function.

Thus by elimination the force necessary for the discharge must be confined to the inner peridial region. We can easily see that in the structure of the palisade layer with its very unusually long, narrow, radially placed cells, free on their inner ends and closely bound together on their outer ends by the layer of small tangential hyphae (fig. 40 and "d" in various figures) we have an ideally constructed mechanism for producing the discharge. The palisade cells if turgid would tend to expand on the inner end while held closely together on the outer end by the tangential filaments. That these cells are extremely, almost explosively, turgid is evident when free-hand sections of fresh materials are cut at the time of the discharge or just after the discharge. Faint pops may even be heard as the cells are cut. This is much more conspicuous in SIII than in the other forms.

The reason for the high turgescence in these palisade cells is due to changes taking place in their cell content. In unopened fruit-bodies, the cells of the palisade layer are densely filled with a granular content (figs. 22, 41, and 50) but after the discharge no cell content is evident (fig. 48). Microchemical tests[5] show that these cells before opening are densely filled with glycogen, that during the morning hours, under the influence of light and heat, the glycogen is transformed into sugars, largely maltose, as the author has previously reported (12). These sugars diffuse into the water that accumulates around the glebal mass. As is well known glycogen exerts almost no osmotic pressure but the osmotic pressure of maltose is extremely high. It is evident that the osmotic pressure developed in the palisade cells, due to the transformation of glycogen to sugars, causes the inner peridial layer to invert and eject the glebal mass.

Distance of Discharge. The force of the discharge is shown by the

[5] The writer is indebted to her colleague, Miss Emma N. Andersen for assistance in making these tests. See Walker and Andersen (12).

distances to which the glebal mass may be hurled. Since SII never forms basidiocarps abundantly no adequate tests have been made as to the distance to which it could hurl its glebal mass nor were any conducted for SIV, but fairly adequate tests have been made for the other forms. The glebal masses, being surrounded by cells that have gelatinized to allow the rupture between the peridial and glebal parts, are so sticky that they adhere to whatever they hit. This has made it possible to test the height to which the glebal masses were hurled.

In order to maintain the proper conditions of humidity a cylinder about 9 inches in diameter and 20 feet long was constructed from sheet celluloid and hung from the railing of a third floor stairway. Cultures were placed on the first floor and allowed to discharge into the tube. As the stairway is so situated that direct light could not reach it a 100 Watt electric globe and reflector were used for illumination. The conditions were far from ideal but the following results were obtained from cultures left in an east window until just before beginning to discharge and then placed under the tube:

HEIGHT OF DISCHARGE	NUMBER OF GLEBAL MASSES		
	SI	SII	SIII
meters			
4 –4½	2		
3½–4	9		3
3 –3½	11	2	4
2½–3	32	10	5
2 –2½	55	16	8
1½–2	82	29	10
1 –1½	122	32	10
0 –1	No count	No count	No count

The large number of glebal masses found in the lower meters is due in all probability to the fact that relatively few fruit-bodies, as formed in flask cultures, point vertically. As can be seen in figures 13–14 the morphological apex of the basidiocarps may be to one side of the top. The reasons for this will be later discussed. No extensive experiments were carried on to determine the horizontal ranges for these forms but a horizontal range of somewhat over 5 meters was secured for both SI and SIII. A greater horizontal range was reported by Dr. A. H. R. Buller at the Chicago meeting of the A. A. A. S. where the writer reported the above results. The writer's experiments on the discharge of SI were previously reported (11).

A comparison with data given by other workers for other forms shows that *Sphaerobolus* is capable of hurling its spore containing mass about five times the distance previously reported. That this result may be expected has been pointed out by Buller (2). He has shown that in all known cases where spores or spore-masses are discharged by fungi those having the larger spore-masses hurl them to a much greater distance than those having smaller masses.

Light Relations of Sphaerobolus. These have been found to be very definite. Cultures kept in the dark or very diffuse light produced no basidiocarps. A vigorous production of fruit-bodies was never secured in cultures except when kept in an east window. During the early stages of my observations it appeared probable that light had no effect upon the direction of the discharge as fruit-bodies side by side were often found opening and discharging their glebal masses in opposite directions. Buller in unpublished work previously mentioned showed a positive heliotropic reaction and experiments conducted by the writer entirely confirm his results. That fruit-bodies in flask cultures point in various directions is due to reflections and refractions of light in the culture flask. When cultures were placed several feet from a window and arranged so that no reflections of light were possible all basidiocarps formed pointed directly toward the source of the light. The heliotropic response is limited to very young basidiocarps. The direction of the light falling upon the maturing fruit-body does not affect the direction of the discharge.

Various attempts were made to illuminate cultures through small openings, using reflectors, and so to develop basidiocarps that would point toward a definite point. All such experiments were unsuccessful because fruit-bodies failed to develop or only a few abnormal ones were developed.

DEVELOPMENT OF SPHAEROBOLUS III

The development of the structures present in the mature fruit-body is of considerable interest. Since the developmental stages are much more easily traced in certain stages in SIII it will be described first and followed by descriptions of development in SIV, SI, and SII.

Origin and Early Stages of Differentiation. The first traces of basidiocarp development consists in the appearance of rounded knots of hyphae that stain more densely than the ordinary hyphae. Two of these are shown (fig. 6 and to right of fruit-body in fig. 16) lying in among the outer peridial parts of discharged fruit-bodies. The fila-

ments in the knot are about 1.5μ in diameter and occasionally show more or less rounded, intermycelial spaces. The hyphae in the knots are binucleate as are also those of the vegetative hyphae. Pillay (9) reports the presence of stromata associated with the development of similar knots in his *Sphaerobolus*. These have never been observed by the writer for any of the forms studied. Pillay (9) obtained his materials from an old wooden, orchid basket in the greenhouse. The very different atmospheric conditions in a greenhouse and in culture flasks might result in greater protection of the initials of the basidiocarp so exposed even if he had worked with the same form. Knots formed among or within old peridia often appeared much like Pillay's illustrations.

The first differentiation of the knot (fig. 7) is the formation of a spherical region of more densely interwoven hyphae surrounding a central region, glebal primordium, which retains the characteristics of the undifferentiated knot. The outer limits of the fruit-body are also more definitely defined, thus marking the limits of the regions that will develop the peridium. In slightly older fruit-bodies (fig. 8) the peridial and glebal regions become very clearly defined. The glebal region includes most of the deeply stained inner zone of densely staining hyphae and all of the parts within. The peridium will develop from the parts to the outside of this zone.

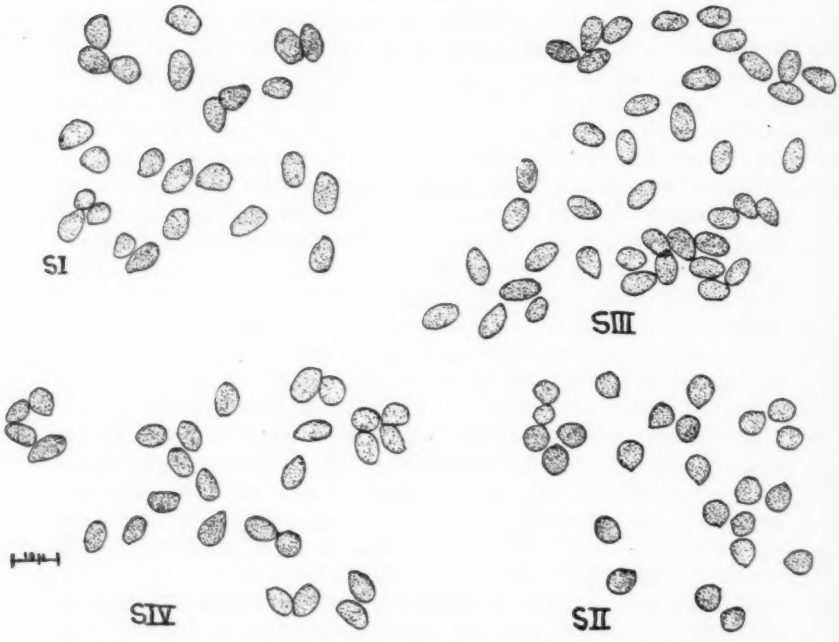
Development of Glebal Mass. The first differentiation discernible in the region that will form the gleba is the presence of much enlarged cells or hyphal ends. The protoplasm in these cells soon becomes much less dense than that of the actively growing cells of the surrounding hyphae and they appear as large empty cells scattered through the region. Figure 8 shows a basidiocarp at this stage. Figure 17 is a higher magnification of a portion of the same basidiocarp and plate 23, figures 1 and 2 show details of these large cells. The smaller of these large cells seem to have two nuclei as do all other cells in the basidiocarp. As these cells enlarge a fusion of the two nuclei occurs and often a large nucleus apparently showing a double spireme is present. Following this the nuclear conditions could rarely be definitely determined because of poor fixation.[6] As the development of these cells seems in all ways like that of the basidia which develop later I conclude that they are at least potentially basidia and shall refer to them as primary basidia. These are undoubtedly the cystidia of other workers. Around

[6] Pillay (9) reports similar difficulties in determining nuclear content in the form studied by him.

these primary basidia with scanty protoplasm many enlarging ends of binucleate hyphae, densely packed with protoplasm, begin to form (pl. 23, fig. 2). They are young basidia. In many cases they soon form an almost complete circle around one or more of the primary basidia. At times the ends of the young basidia are seemingly almost flattened by pressure against a central primary basidium. In other cases the young basidia may similarly group themselves around a central space where there is no primary basidium so that the presence of the primary basidium does not seem essential for the development and orientation of basidia. Figures 9 and 10 show fruit-bodies only slightly older than the one shown in figure 8. As the basidia enlarge very definite glebal chambers surrounded by a palisade-like layer of basidia result. In some places remnants of primary basidia may be seen. Figures 19 and plate 23, figure 3, show details from figure 9. As development progresses new chambers arise in the same manner as the first chambers developed, especially toward the outer portions of the gleba. As the basidia increase in number the glebal chambers enlarge (figs. 10, 11, 13, and 14) until quite sizable chambers are present. The basidia begin to form spores very soon after their formation, so that in a fruit-body only very slightly older than shown in figure 10 individual basidia with spores may be found in almost every chamber. Plate 23, figure 4, shows the arrangement of the basidia in the chambers and the nature of the tramal parts at this stage. An individual basidium with spores from an adjoining chamber is shown at the right. In figure 11 a basidiocarp is shown in which spore development is abundant but very few mature spores are present. Successively older stages are shown in figures 13 and 14. As the spores mature, 6–8 being formed on each basidium, the chambers gradually become filled with spores as may be seen in the lower parts of the basidiocarps shown in figures 13–15. In the upper parts of the same fruit-bodies is seen the packed condition of spores which are still adhering to the basidia. Figure 18 illustrates a portion of the gleba from the upper part of the basidiocarp shown in figure 15. Practically all of the contents of the older basidia has passed out into spores and in places the basidia are beginning to break down but new basidia are still forming among the old ones. Plate 23, figure 5, shows the details of one of the smaller chambers. In the center, sections of spores from basidia below are outlined.

All of the stages mentioned above and illustrated have been repeatedly observed also in free-hand sections of fresh material. A comparison of the fresh materials with the stained sections has been very helpful,

especially in distinguishing normally developed basidiocarps from depauperate ones which were constantly encountered. Depauperate fruit-bodies frequently enlarge and even discharge their glebal masses apparently in an entirely normal fashion. Such basidiocarps have been observed where glebal development had been arrested at a stage as young at least as in the specimen shown in figure 8. In free-hand



Text-fig. 1. Spores from dried glebal masses of the four strains of *Sphaerobolus* drawn to the same scale.

sections the gleba of such basidiocarps is watery and transparent; that of vigorous normally developed fruit-bodies is white and more or less opaque. In paraffin sections these depauperate specimens were often helpful in verifying observations on younger basidiocarps.

The nuclear behavior during the development of the basidia could not be followed as well in this form as in SIV. This is doubtless due to the greater amount of slime present in the gleba which prevented proper

fixation. In younger basidiocarps and near the periphery of older basidiocarps fair fixation was secured. So far as observed the development was like that of SIV which will be described in greater detail. Young spores are definitely uninucleate and older spores appear to be binucleate.

Soon after the development of the spores the basidia break down and the tramal hyphae enlarge, apparently gelatinizing. The interior of a nearly mature glebal mass consists largely of spores held in the meshes of hyphae which have become 4–6μ in diameter (figs. 16 and 20). Mature spores are oval to slightly pointed at one end. Spores from discharged glebal masses are shown in text-fig. 1.

The enlargement of the tramal hyphae is followed by their complete disintegration. At a stage shortly before discharge of the glebal mass free-hand sections show the tramal parts reduced to a gelatinous appearing reticulum from the meshes of which the spores drop out readily. At the time of the discharge (fig. 12) many spores will still separate from the slime developed from the more completely disintegrated tramal parts. After the glebal mass has been discharged a few hours it is almost impossible, in water mounts, to separate spores from their enveloping slime. The slime appears as an oily, gluey mass that sticks tenaciously to any dry object. The contents of the gleba remain soft and gluey even after years of storage in a dry laboratory.

Some study has been made upon the development of the oily slime that eventually envelops the spores. Many observations and tests were made upon basidiocarps in various stages of development but with unsatisfactory results. Oil[7] was never observed in the basidia at any stage of their development. There is always an abundance of oil in the tramal hyphae at stages as young as shown in figure 8. As the hyphae disintegrate in older stages oil globules often appear to be on the outside of the gelatinizing hyphae. In still older stages masses of oil globules are all that may be seen where the tramal hyphae have been. The slime found mixed with the spores in discharged glebal masses consists largely of oil. The oil seems to be mixed with a more or less gelatinous substance which may be pectic. Granules and much broken down remnants of tramal parts are also present. The oil may be readily extracted by treatment with ether, xylol, or absolute alcohol. When ether is applied to smears from glebal masses the spores are set entirely

[7] Osmic acid never gave satisfactory tests for the oil present even when applied to smears from dried glebal masses. For this reason Sudan III was used exclusively.

free. When similar smears are treated with xylol most of the spores remain in the smear after the oil has been extracted.

At the time the basidia begin their development the hyphae on the outside of the glebal mass become gradually enlarged and massed together to form a definite pseudoparenchyma (figs. 11, 13–15) until at maturity ("F" in figs. 21 and 22) it consists of large, heavy walled, brown cells, 6–18μ (average 10μ) in diameter. The pseudoparenchyma inclosing the gleba forms a much tougher membrane than in the other forms studied so that the discharged glebal mass appears like a bag of glue. Within the pseudoparenchyma layer bordering the gleba (figs. 21 and 22) occur many large cells with densely staining contents. These are the so-called gemmae of other workers. Many of these have the appearance of basidium-like cells. Others are irregular in shape and intercalary. In mounts they very commonly appear as two-celled structures. Just as in other described species of *Sphaerobolus* large crystals are also conspicuously present in the gleba when examined in fresh materials.

Development of Peridium. As was mentioned before, the peridial parts at first consist of undifferentiated, interwoven hyphae. Plate 24 shows successive stages in the development of the peridium in the lower portions of fruit-bodies. The details of the peridial differentiation seen in figure 8 are shown on plate 24, figure 1. The filaments show practically no differentiation but are massed together in two regions "f" and "e" and from these the two layers of pseudoparenchyma seen in the older basidiocarps will develop. In the region "e" the filaments have taken a somewhat radial direction and are the filaments which will form the palisade-like layer in the mature fruit-body, while in "d" a somewhat tangential position of the hyphae distinguishes the region of tangential hyphae bordering the palisade in the mature basidiocarp. The region "a" undergoes but little differentiation during development. The cells enlarge somewhat and the walls become firmer. In the filamentous parts "a" and "d" some hyphae enlarge greatly and become densely filled with a deeply staining granular content, probably glycogen. As they mature (pl. 24, fig. 4) these appear much like the so-called gemmae in the glebal region and like them are stippled where shown. The successive stages in the development of these peridial parts may be seen in figures 11, 13–16. Plate 24, figure 2, shows the detail of the peridium of the fruit-body seen in figure 11; plate 24, figure 3, of the fruit-body in figure 13; and plate 24, figure 4, of the fruit-body in figure 16. The detail of the lower peridium of a nearly mature basidiocarp is shown in figure 22.

In the lower portions of the basidiocarps shown in figures 11, 14, and 22 it will be observed that the palisade layer and the layer of pseudoparenchyma bordering the gleba are interrupted by strands of hyphae that pass directly from the filamentous layer ("d" in fig. 22) into the interior of the glebal mass. Several, usually 5–7, of these strands are formed through these layers and maintain vital nutritive paths of connection between exterior and interior parts of the basidiocarps. Under a dissecting binocular microscope these strands appear as definite rounded pore-like spots in the palisade layer after the discharge of the glebal mass.

Toward the upper portion of the fruit-body the cells of the palisade layer become shorter and gradually pass over into a pseudoparenchymatic condition at the top. The other layers practically remain the same in all parts (figs. 11, 13–16). Figure 21 is a higher magnification of the upper part of the fruit-body shown in figure 16. It is through this region where the palisade layer has been transformed into pseudoparenchyma that the stellate rupturing of the peridial parts takes place in the mature fruit-body. This rupturing of the peridium extends from the outside to the border line between "e" and "f" on plate 24, figure 4. The gelatinization of these bordering cells takes place first in the upper part of a fruit-body so that in the morning when a basidiocarp first opens the glebal mass is usually firmly anchored to the base of the peridium. In a very brief time the gelatinization in this region completely surrounds the glebal mass. In the meantime gelatinization is also taking place in the region where the pseudoparenchymatic layer "c" joins the layer of tangential hyphae "d," as seen in figure 22 and plate 24, figure 4. This gelatinization begins at the base of the basidiocarp and progresses upward. Before the discharge takes place these layers are so completely separated that the point of a needle may be inserted between the two layers in the sinuses of the notches (pl. 22, above). This separation is so complete before the discharge that the palisade and tangential layers ("d" and "e" in fig. 22), making up the inner peridial wall, are usually forcibly ejected along with the glebal mass, often to a meter in distance. The regions of separation are clearly seen in figure 12.

DEVELOPMENT OF SIV (S. STELLATUS)

The three remaining forms (SI, SII, and SIV) are very similar in structure and development. The development of the European form

(SIV) isolated by Kniep will be described in detail and variations in the other strains mentioned.

Origin of Basidiocarp. The first traces of basidiocarp development observed consist of vaguely globular knots of hyphae that are smaller and stain more densely than those of the surrounding hyphae. These meristematic hyphae are very similar to rapidly growing vegetative hyphae and it is only when a layer in the periphery of the mass begins to gelatinize that a definitely formed primordium of a basidiocarp is evident. Figures 23–26 show young fruit bodies at this stage of development. In this form also there was no evidence of a stroma such as described by Pillay (9). These knots vary considerably in appearance but the gelatinous layer is always thicker on the upper surface. On the lower side it is always interrupted by strands of active hyphae (figs. 23–25) which connect with vegetative hyphae. In many cases the gelatinous layer may be wanting or weakly developed on the lower surface. The young basidiocarps and the mycelium giving rise to them are binucleate. The nuclear behavior during the development of the fruit-bodies could be much more definitely determined in SIV than in the other forms. So far as could be observed the nuclear condition was very similar in all four strains. The nuclei in young basidiocarps are about $1\frac{1}{2}\mu$ in diameter and, in all regions, except the jelly layer, completely fill the diameter of the cell. In some cases the hyphae were only about 1μ in diameter and the nuclei were laterally compressed. In the jelly layer the hyphae are slightly broader, about 2μ in diameter. The central portion takes the stain much more deeply because of denser cytoplasm and more numerous nuclei. The cells also seem to be shorter but the hyphae of the central region are so densely interwoven it was impossible to determine the length of the cells. Figure 37 is a higher magnification of the central portion of the basidiocarp shown in figure 25. The basidiocarp shown in figure 26 is slightly older. This is clearly indicated by the presence of hyphal differentiation in the interior. Instead of hyphae of uniform size there are found larger and smaller hyphae with variable protoplasmic content. A few of the hyphae at the center have clavately enlarged ends, measuring up to 2.5μ in diameter, which are densely filled with protoplasm. These enlarged cells are the beginnings of the first basidia and appear as shown on plate 25, figure 1. The peridium is entirely undifferentiated at this stage except for the gelatinized zone in the outer portion which was the first differentiation to take place in the young mycelial knot.

The thickness of the gelatinous layer at all stages in the development

of the basidiocarp is extremely variable. Figure 27 shows a basidiocarp only slightly older than the one seen in figure 26 and decidedly younger than the one seen in figure 28. Similar variations are shown in figures 29, 30, and 33 where the fruit-bodies are not of very different age.

Development of Gleba. Following the appearance of enlarged cells (young basidia) in the center of the basidiocarp referred to above, basidia multiply conspicuously and characteristically. The first basidia or primary basidia become centers around which young basidia center and as a result we have clusters of densely stained hyphae interspersed with sterile hyphae (fig. 28). The details of the gleba at this stage are somewhat diagrammatically shown on plate 25, figure 2. Thus at this early age the beginnings of the sterile and fertile parts of the gleba are clearly differentiated. The primary basidia are now about 5μ in diameter.

These central basidia and similar cells that develop later have been considered cystidia by earlier workers and by the writer at first because of their scanty protoplasmic contents. Further studies show that each contains a large fusion nucleus by the time it has reached this age. The development of these basidia could not be traced in normal healthy basidiocarps but in depauperate fruit-bodies it was very clear that these develop basidiospores abundantly. As the spore-bearing basidia grow older they too cease to take stains deeply and present the same appearance as the primary basidia.

As development progresses additional spore-bearing regions are initiated in the same manner as the original ones just described. These develop more abundantly in the peripheral portion of the gleba but may arise in any portion. It is also evident that some spore-bearing regions arise without the presence of primary basidia as in some cases none could be located. There is however a fairly definite orientation of the young basidia around a common center.

Figure 29 shows a basidiocarp somewhat older than the one shown in figure 28. The gleba in figure 29 extends to the inner portion of the deeply staining zone that has developed in the basidiocarp. A higher magnification of the central part of this basidiocarp is shown in figure 36. By this time the primary basidia have attained a diameter of about 7μ. The details of one fertile region are shown on plate 25, figure 3. It is possible that this fruit-body is slightly depauperate.

The basidiocarp shown in figure 33 is very different in appearance but only slightly older as indicated by a slight enlargement of the hyphal ends surrounding the primary basidia. In figure 30, more highly mag-

nified in figure 38, is shown a still older basidiocarp. The hyphal ends have enlarged and crowded around the central cells and in many cases have entirely obliterated them. The primary basidia are uninucleate but most of the encircling young basidia are binucleate where the nuclear condition could be observed. The appearance of the gleba at about this stage is shown on plate 25, figure 4. The inner portion of the densely staining zone surrounding the gleba is also massing together preparatory to forming the pseudoparenchyma of the wall of the glebal mass. It still, however, retains a filamentous structure.

The fruit-body shown in figure 32 marks a decided advance. The primary basidia in the spore-bearing regions have almost entirely disappeared or are not distinguishable from the basidia developed later. A definite orientation is seen in many cases but in other cases the basidia have become so crowded that no orientation is observable. This may be definitely seen in a more highly magnified portion of the gleba shown in figure 39. Most of the basidia at this stage show one large nucleus but young binucleate basidia are commonly observed. In a few basidia the primary nucleus of the basidium seems to have divided to form the nuclei that will pass into the spores but the nuclei at this stage are so difficult to distinguish that it is impossible to be positive. The details of a small region near the center of this fruit-body are shown on plate 25, figure 5. No spore formation was observed. In a slightly older basidiocarp, such as is shown in figure 31, a few of the basidia are beginning to form spores. In still older basidiocarps (fig. 34) basidia in all stages of development are to be found.

The basidia, as has been mentioned before, are at first binucleate. These nuclei fuse and form one large nucleus which persists for a relatively long period as is the case in most basidiomycetes. The first and second mitoses seem to be normal and give rise to four nucleate basidia. In many cases only four nuclei could be found in basidia that had well developed spore walls but in other cases eight nuclei, of about half the size of those found when four nuclei were present, were observed. As the basidia mature the nuclear membrane disappears and nuclear granules appear at the apex of the basidium. One of these granules passes into each of the spore walls and becomes the primary nucleus of the spore. Where less than eight spores develop on a basidium nuclear granules remain in the basidium after the others have passed out into the spores. The spores remain uninucleate for some time but many old spores seen in mature basidiocarps have two nuclei. The

basidia entirely break down and disappear as soon as the spores are matured, making room for the enlargement and maturation of other basidia. The writer's observations on the development of the basidia agree in general with those of Pillay (9). The most striking variation in our observations is the length of the period elapsing between fusion and reduction division. He says that the fusion nucleus shows no resting stage but passes at once into reduction division. In all four forms examined in these studies the fusion nucleus persists a considerable time and is the most conspicuous stage observed. Pillay's interpretations were somewhat complicated by the presence of nine-spored basidia in the form studied by him. In no case in stained sections or in fresh mounts were more than eight-spored basidia ever observed in any of the forms studied. Much more study should be given to the development of these basidia before a satisfactory account of their development is possible.

In a nearly mature basidiocarp as shown in figure 35 we find spore-bearing regions and much enlarged sterile hyphae and gemmae intermingled in the gleba. These appear like those of SI as shown in figure 46. The sterile hyphae are undergoing disintegration and become almost completely broken down by the time the glebal mass is discharged. The gemmae are more or less scattered through the glebal mass but are more abundant toward the periphery. They are basidium-like cells in form but have a much more densely granular content than the basidia. Two or more nuclei were observed in each gemma. The mature spores (text-fig. 1) are more or less elongated, rounded at one end, and taper to a slight apiculus at the other.

In this form also the broken down tramal parts seem to be transformed almost wholly into oil. Dried glebal masses, however, become firm and hard and lose the gluey consistency so characteristic of SIII.

Development of Peridium. The peridium of SIV follows almost exactly the same sequence in development as described for SIII except the interpolation of the jelly layer in the outer peridial parts. The structure of this jelly layer shows well at the left side of the young basidiocarp shown in figure 38 and may be seen less clearly in the outer parts (b) of figures 41 and 42. As the basidiocarp matures the jelly layer becomes smaller and smaller till it entirely disappears by the time the glebal mass is discharged. This may be seen in figs. 49 and 48 taken from SI which is identical in structure with SIV.

VARIATIONS IN SI AND SII

As SI and SIV grow side by side they are quite easily distinguishable. The mature basidiocarps of SIV are smaller and do not open as widely as those of SI. The glebal mass of SIV is also somewhat darker. In fact SIV looks more like SIII as it grows than SI. SIV occasionally fruits on agar-agar cultures but SI never has. Both fruit equally readily and abundantly on sawdust, dung, etc. The origin and development of basidiocarps are so similar that sections of one might be substituted in a series for those of the other. SI seems a little more vigorous than SIV and the gleba is more densely packed with basidia or spores owing to the age of the specimen. A few sections are shown for the sake of comparison. The basidiocarp shown to the right in figure 43 is intermediate between those of SIV shown in figures 28 and 29; the one to the right in figure 43, about the age of SIV shown in figure 33. The jelly layer did not take the stain well on this slide and is rather indistinct on the upper side. The details of early stages in the development of SI are shown on plate 23, figures 6–10. Figure 6 (pl. 23) is taken from a basidiocarp showing a very early stage in glebal differentiation. The enlarged cells are young basidia. Plate 23, figure 7, shows a little of the gleba from the younger basidiocarp shown in figure 43 and figure 8 (pl. 23) is from a slightly older fruit-body. Details from a basidiocarp about the age of the older basidiocarp shown in figure 43 are shown on plate 23, figures 9 and 10. Some fertile regions show large cells in the center (fig. 9) but in other cases no such cells could be found (fig. 10). Figures 45, 44, and 49 show successive stages in development of the basidiocarp. Figure 49 also shows an unusual case where the gleba is depauperate at the base in an otherwise normal fruit body. Figure 48 shows the peridium after the discharge. The structure of outer portions of the nearly mature basidiocarps are shown in figures 47 (at apex) and 40 (at base). The lettering corresponds to that used for SIII and SIV. There is much more of the oily slime in older basidiocarps and discharged glebal masses of SI than SIV. The spores of the two strains are identical (text-fig. 1). SI and SIV are so similar and the variations of such a secondary nature as should be expected in two strains of the same species.

SII has, as was previously stated under "materials," a basidiocarp about twice the size of that of the other strains of *Sphaerobolus*. It fruits scantily and only in older cultures at higher temperatures. Its spores are more globular than those of the other species but similar to

the more globular spores that are always found among the spores of SI and SIV (text-fig. 1). Externally it looks like a giant SI, internally the gleba is less packed with basidia and spores like SIV. The discharged glebal masses are very soft and flatten against any hard surface it hits; those of the other forms are turgid and remain spherical. SII is, however, so similar to *S. stellatus*, in all stages of its development, that it is only distinguishable by the larger size of its basidiocarps and by the entire lack of elongated spores. The larger size of the basidiocarps is evident from the origin of the fruit-body in a larger tangle of hyphae up to the mature basidiocarp. It seems best therefore to consider it as only a variety of *S. stellatus*.

DESCRIPTION OF NEW FORMS

Because of the large size and characteristically different spores of SII, the following new variety is proposed:

Sphaerobolus stellatus var. **giganteus** n. var.

Basidiocarps having the color, structure, and appearance of *S. stellatus* but being much larger—up to 4 mm. in diameter (usually about 3 mm.) of unopened basidiocarps and 5–6 mm. from tip to tip of peridium in opened basidiocarps. Spores globular with a slight apiculus, 5–7 x 6–8μ, mostly 6 x 7μ.

Type locality: Starkville, Miss., on dung (horse?).

Distribution: Known only from type locality.

Type specimens: Herbarium University of Nebraska.

A comparison of SIII with SI, SII, and SIV shows that SIII differs from the other strains very markedly in both glebal and peridial structures. The gleba is characterized by the presence of conspicuous chambers during its developmental stages and the peridium by the entire absence of a gelatinous layer in its outer peridium. These structures are so fundamentally different that SIII must be considered a distinct species. The following specific name and characterization are proposed:

Sphaerobolus iowensis n. sp.

Unopened basidiocarps 1–1½ mm. in diameter. Peridium breaking stellately at the apex into 3–8 parts (usually 4–5), the tips becoming only slightly recurved so that the peridium of the opened fruit-body is somewhat cup-shaped. Interior of peridium cadmium yellow when first opened, glebal mass raw umber[3] when first exposed, soon becoming almost black. Peridium separating into two regions, an outer and an

[3] See Ridgway, *Color Standards and Nomenclature*.

inner. Outer peridium during its development and at maturity composed of two layers, an outer filamentous and an inner pseudoparenchymatic. Inner region of a layer of tangential hyphae and a palisade layer. Gleba containing during its development many definite chambers lined with basidia. Wall of glebal mass firm, composed of cells 6–15μ in diameter. Contents of glebal mass soft and gluey, never drying hard. Spores oval, 5–6 $\times$ 6–10μ (usually 5.6 $\times$ 8μ).

Type locality: Hunters, Iowa, on old coniferous boards.

Distribution: Known only from type locality.

Type specimens: Herbarium University of Nebraska.

Distinguishable from *S. stellatus* by its chambered gleba and the absence of a gelatinous layer in the peridium.

MISCELLANEOUS OBSERVATIONS

Germination of spores and gemmae. Scores of hanging drops have been made from the spores of SI, SII, and SIII in an attempt to determine whether these forms were homothallic or heterothallic. The gemmae of all three strains germinate readily and within 24 hours give rise to hyphae with abundant clamp connections but spore germinations were rarely secured. The abundance of gemmae mixed with the spores and their ready germination made observations on spore germination very difficult. Hanging drops were made in water and in various nutrient media but spore germinations were obtained only in water to which a trace of pepsin had been added, a method used by Pillay (9). Even in these cases spore germination was very scanty and uncertain. The scarcity of spore germinations has made one-spore cultures practically impossible.

In SI the smaller gemmae often so closely resemble spores as to make it almost impossible in all cases to distinguish them positively. The spores have a thicker, more highly refractive wall than the gemmae but could only be distinguished with a fair degree of accuracy after germination. Cells that had the appearance of spores gave rise to germ tubes with abundant clamp connections thus indicating that this strain is diploid or homothallic as Kniep (5 and 6), Bensaude (1), Mounce (7 and 8), and later workers have shown. Pillay (9) made extensive germination cultures of the *Sphaerobolus* studied by him with the same conclusion.

Spores of SII can be very positively distinguished from gemmae by their smaller size. The spores usually give rise to only one germ tube but two or three germ tubes are not uncommon. The germ tube may

remain almost unbranched or branch profusely. No clamp connections have ever been found on germ tubes but neither were cross walls observed. The behavior of the germ tubes is interesting in that in many cases they intermingled seemingly with no tendency to anastomose but in other cases abundant anastomoses occurred. In a few cases clamps were observed on older hyphae after much anastomosing. The germ tubes could not be accurately followed as they intermingled and it was impossible to be sure whether or not clamps occurred if anastomoses did not occur. Fischer (3) describes the spore germination of his *S. stellatus* as having one germ tube which rarely develops cross walls.

Spore germinations from SIII (*S. iowensis*) were obtained in only one instance. The germ tubes were much longer and slender and branched much less than those coming from gemmae. The cross walls were far apart. Clamps were usually absent at the cross walls but in one instance a clamp was clearly seen. It is probably homothallic.

Vitality of Glebal Masses. All cultures used in these studies were obtained from discharged glebal masses. Fischer (3) and Pillay (9) have determined that the hyphae emerging from the glebal masses come from gemmae. I have made no detailed studies but the mycelium emerging from the glebal masses is always abundantly provided with clamp connections like that from gemmae. If this is true the gemmae must be extremely long-lived. Old culture flasks have been saved and fresh cultures started from glebal masses of SI that had been discharged at least seven years previously. Growth started more slowly but the cultures produced were as vigorous as those started from fresh material.

TAXONOMIC CONSIDERATIONS

Rabinowitsch (10) and Pillay (9) have pointed out the fact that the strains of *Sphaerobolus stellatus* studied by them had a definite arrangement of the basidia in the gleba instead of the entirely scattered basidia characteristic of some of the Sclerodermatales (Plectobasidiales). Because of the entire absence of definite glebal chambers, however, they have considered *Sphaerobolus* more closely related to the Sclerodermatales than to the hymenium bearing Gasteromycetes such as the Lycoperdales but suggest a possible intermediate relationship.

The very evident glebal chambers in *S. iowensis* (SIII), which persist up to maturity and are very evident even in sections of dried, unopened basidiocarps, point directly to a relationship with the Lycoperdales. The chambers of *S. iowensis* are smaller but fully as definite as in species

of *Geaster*, *Lycoperdon*, and *Bovista* sectioned by the writer for comparison. The chambers of *S. iowensis* are, in fact, fully as large in comparison to the size of the basidiocarps as in the forms mentioned above. In *S. stellatus* (SI, SII, and SIV), however, true glebal chambers are never present and an orientation of the basidia is seen only in very young stages. In many cases no definite orientation was observed. The very earliest stages in glebal development are alike in all four forms and the development is fundamentally alike in all. In *S. stellatus* the orientation of the basidia is less definite and the central space is soon filled with developing basidia and entirely obliterated but in *S. iowensis* the orientation is definite and the interpolation of new basidia causes the enlargement of the hymenium and the formation of definite chambers.

According to the most commonly accepted classification of Gasteromycetes, that given by Fischer (4), the orders Lycoperdales and Sclerodermatales are separated primarily on the presence or absence of glebal chambers in the development of the basidiocarps. An adherence to this has led to the formation of the genus *Astraeus* for our well known *Geaster hygrometricus* and it has been placed in the Sclerodermatales, far from the geasters to which it is so closely related by appearance. Similarly an adherence to this characteristic would place *S. iowensis* in the Lycoperdales and *S. stellatus* in the Sclerodermatales. This would, however, be entirely improper because it would again separate forms that are obviously closely related. The mature gleba of the Lycoperdales is characterized by an abundant development of capillitium in the meshes of which powdery spores are held. It seems to the writer that this should be taken as the most important characteristic of the order. This would allow forms like *Geaster hygrometricus*, *Tylostoma*, etc., to be transferred to the Lycoperdales which they more closely resemble. The order Sclerodermatales might be retained for terrestrial forms that lack a well developed capillitium and retain a fleshy gleba until mature.

The writer believes that further studies upon the development of the gleba in forms now placed in the Sclerodermatales are necessary before we can have a clear idea as to whether or not there are fundamental differences between Plectobasidiomycetes and Hymenobasidiomycetes.

SUMMARY

1. Four strains of *Sphaerobolus* were grown in cultures and studied during a period of years. Two strains SI and SIV are undoubtedly the

same species, *S. stellatus*. SII is considered a new variety, *S. stellatus* var. *giganteus*, and SIII is described as a new species, *S. iowensis*.

2. *S. iowensis* differs from *S. stellatus* primarily (a) in the entire absence of a gelatinous layer in its peridium and (b) in the presence of conspicuous chambers in the gleba during its developmental stages. A conspicuous gelatinous layer is always present in the other forms studied.

3. The primordia of basidiocarps and earliest developmental stages are very similar in all four forms (except for the absence of the gelatinous layer in *S. iowensis*). A more definite orientation of basidia in *S. iowensis* causes the development of chambers but in the other forms no orientation of basidia is found after the very early stages.

4. Basidium development is very similar in all three forms. The young basidium is binucleate; these two nuclei fuse to form the primary nucleus of the basidium which is a conspicuous stage. The fusion nucleus gives rise successively to 2, 4, and 8 nucleate stages. Five to eight spores are formed on each basidium. The mature spores of all seem to be binucleate.

5. Spore germination was obtained only in water to which pepsin had been added. Germinations indicate that SI and SIII are definitely homothallic; SII was uncertain but possibly homothallic.

6. The energy necessary to produce the forceful discharge of the glebal mass is obtained by a sudden transformation of glycogen to sugars (largely maltose) in the palisade layer shortly before the discharge.

7. The glebal masses may be hurled to a height of over 4 meters, a greater height than is known for any other fungus.

8. The glebal masses retain their vitality at least 7 years.

9. The variations in glebal structure observed suggest that the separation of Gasteromycetes upon the presence or absence of glebal chambers is not advisable and that other criteria should be used for the distinguishing characteristics of orders.

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EXPLANATION OF PLATES

All photographs were made by the author except that shown in figure 2 which was made by Mr. Frank Shoemaker. Detailed explanations of figures may be found in the text.

PLATE 16

SPHAEROBOLUS STELLATUS (SI)

Fig. 1. Young culture in a liter flask.
Fig. 2. Basidiocarps just before discharge; × 5.
Fig. 3 a and b. Two photographs of the same culture; "b" taken 30 minutes after "a"; × 1½.
Fig. 4. Vigorously fruiting culture developed on sawdust in a liter flask. Slightly enlarged.

PLATES 17 AND 18

SPHAEROBOLUS IOWENSIS (SIII)

Fig. 5. Three basidiocarps; × about 1.
Figs. 6–11, 13–16. Median sections of successive stages in the development of basidiocarps; × 34.

Fig. 12. Median section of peridium and glebal mass just before discharge; × 25.
Fig. 17. Part of central portion of section shown in fig. 8, and pl. 23, figs. 1 and 2; × 283.
Fig. 18. Part of gleba from basidiocarp shown in fig. 14; × 283.
Fig. 19. Part of central portion of basiodiocarp shown in figs. 9 and pl. 23, fig. 3; × 283.
Fig. 20. Portion of gleba from basidiocarp shown in fig. 16; × 283.
Figs. 21 and 22. Portions of peridium at apex and base of basidiocarp shown in fig. 16; × 283.

PLATES 19 AND 20

SPHAEROBOLUS STELLATUS (SIV)

Figs. 23–35. Median sections of stages in the development of basidiocarps. Figs. 23–27 are young knots; figs. 28, 29, 33, 30, 32, 31, 34, 35, represent a developmental series; × 37.
Fig. 36. Central portion of basidiocarp shown in figs. 29 and pl. 25, fig. 3; × 283.
Fig. 37. Central portion of basidiocarp shown in fig. 25; × 283.
Fig. 38. Part of basidiocarp shown in fig. 30; × 283.
Fig. 39. Part of gleba of basidiocarp shown in figs. 32 and pl. 25, fig. 5; × 283.
Fig. 40. Tangential section of a mature basidiocarp showing hyphae of thread layer "D" in the basidiocarp shown in figs. 35, 41, 42; × 283.
Figs. 41 and 42. Portions of peridium at apex and base of basidiocarp shown in fig. 35; × 283.

PLATE 21

SPHAEROBOLUS STELLATUS (SI)

Fig. 43. Median sections of two young basidiocarps, younger to right; × 33.
Fig. 44. Practically mature basidiocarp; × 28.
Fig. 45. Nearly mature basidiocarp but considerably younger than one shown in fig. 44; × 33.
Fig. 46. Portion of gleba from a mature basidiocarp; × 293.
Figs. 47 and 50. Portions of peridium from upper and lower parts of a nearly mature basidiocarp; × 293.
Fig. 48. The peridium after the discharge of the glebal mass; × 25.
Fig. 49. Mature basidiocarp just before discharge, depauperate at base. Note disappearance of the gelatinous layer in the peridium; × 28.

PLATE 22

[Above] Mature basidiocarp of a *Sphaerobolus* before discharge (left) and the position of the peridial layers after the discharge (right).
[Below] Arrangement of tissues in the outer portion of a nearly mature basidiocarp of *S. stellatus* (SI). In 1, the figure at the left shows tissues at the apex of a basidiocarp; the figure to the right shows those at the base. Outer peridial portion, a–c; inner peridial portion, d–e; outer portion of glebal region, f–g. Tracings from photographs; × about 300. 2. Outer portion of a discharged glebal mass showing gemmae, spores, and hyphae; × 578.

PLATE 23

Development of gleba in *S. iowensis* (SIII), 1–5, and *S. stellatus* (SI), 6–10. 1 and 2, portions of the gleba from the basidiocarp shown in fig. 8; 3, in fig. 9; 4, in a basidiocarp slightly older than shown in fig. 10; and 5, in fig. 15 of plate 17. Figs. 6–10, successive stages in the development of the gleba up to basidiocarps of the age of the older one shown in fig. 43 of plate 21. Fig. 3 × 1000; fig. 5 × 540; other figs. × 1250.

PLATE 24

Development of peridium near base of basidiocarp in *S. iowensis* (SIII): a–c, outer peridial portion; d–e, inner peridial portion; f–g, outer portion of glebal region. 1, from basidiocarp shown in fig. 8; 2, from basidiocarp shown in fig. 11; 3, from basidiocarp at age of one shown in fig. 14; and 4, from basidiocarp shown in fig. 16, plate 17; × 490.

PLATE 25

Development of gleba in *S. stellatus* (SIV). 1, a portion of the gleba from the basidiocarp shown in fig. 26; 2, glebal structure as seen in fig. 28; 3, as seen in figs. 29 and 36; 4, as seen in a slightly older basidiocarp; and 5, as seen in figs. 32 and 39. All figures × about 2200.

PLATE 16

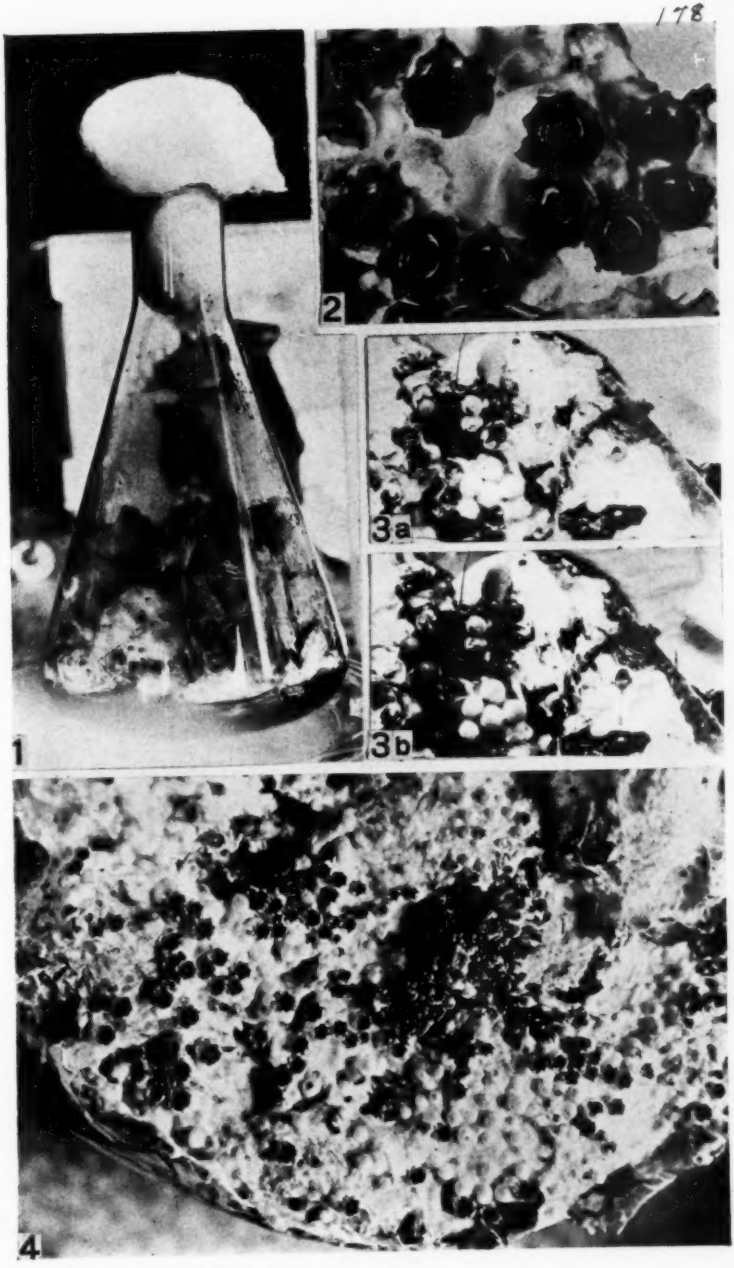

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PLATE 17

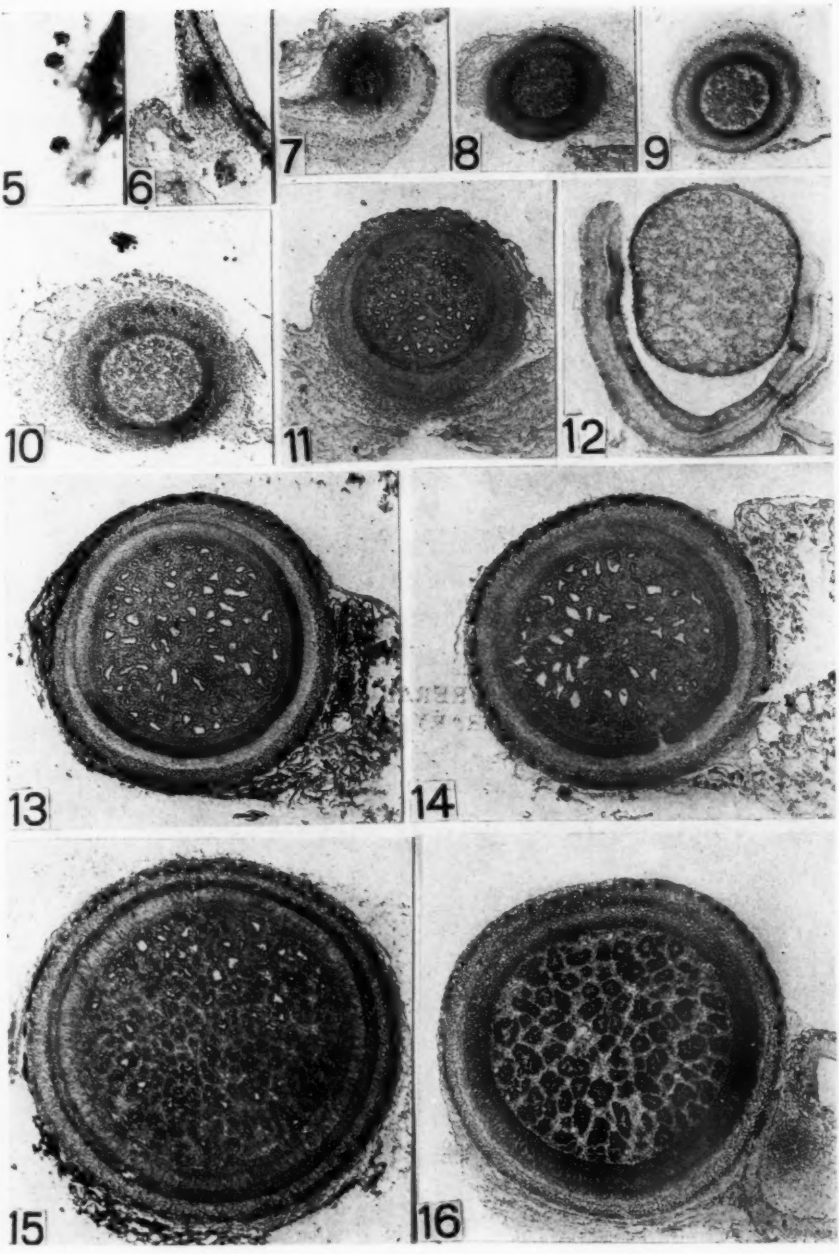

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PLATE 18

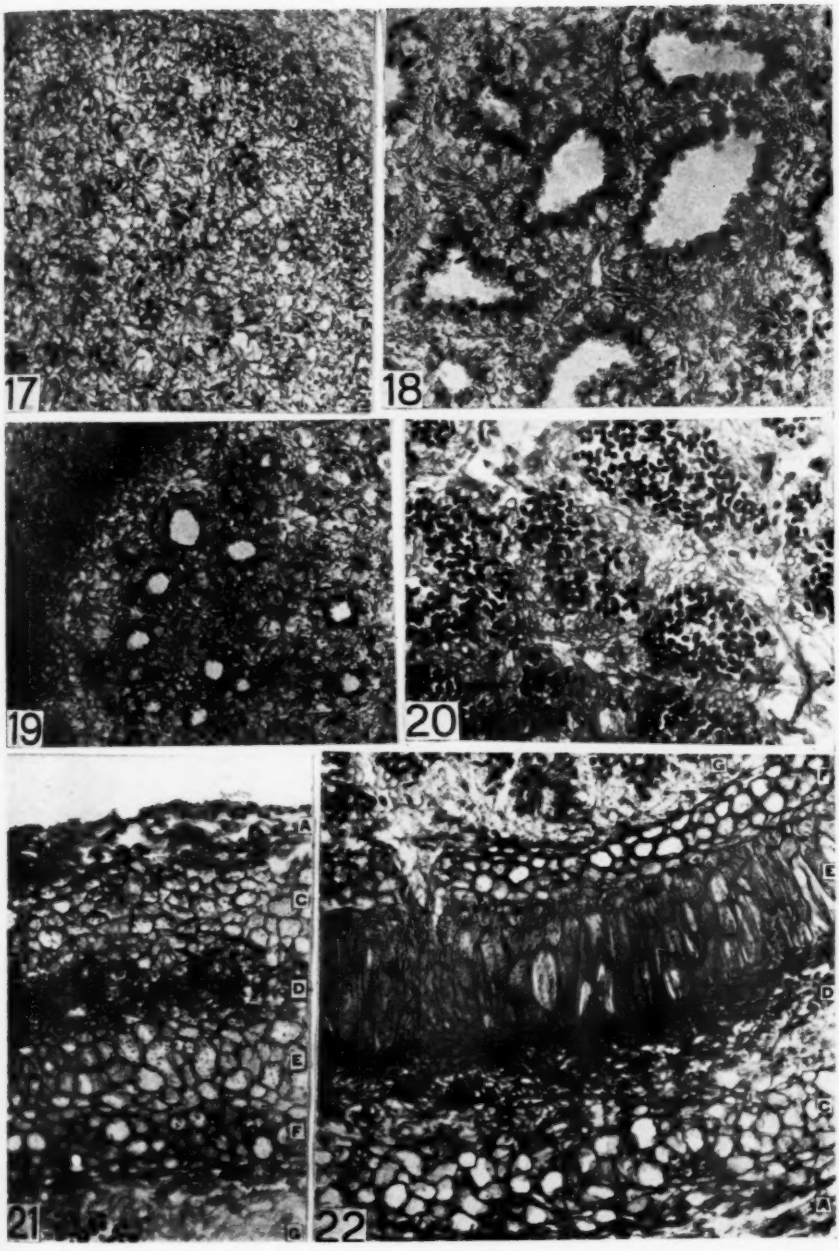

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PLATE 19

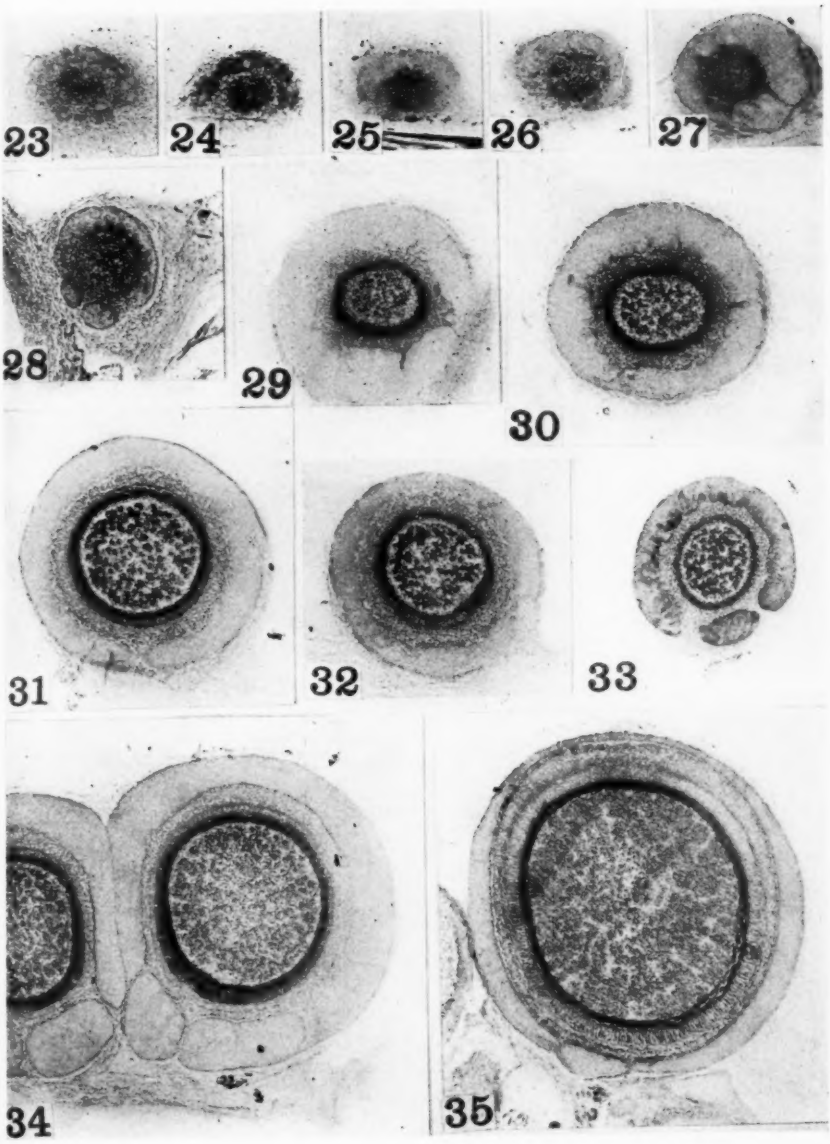

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PLATE 20

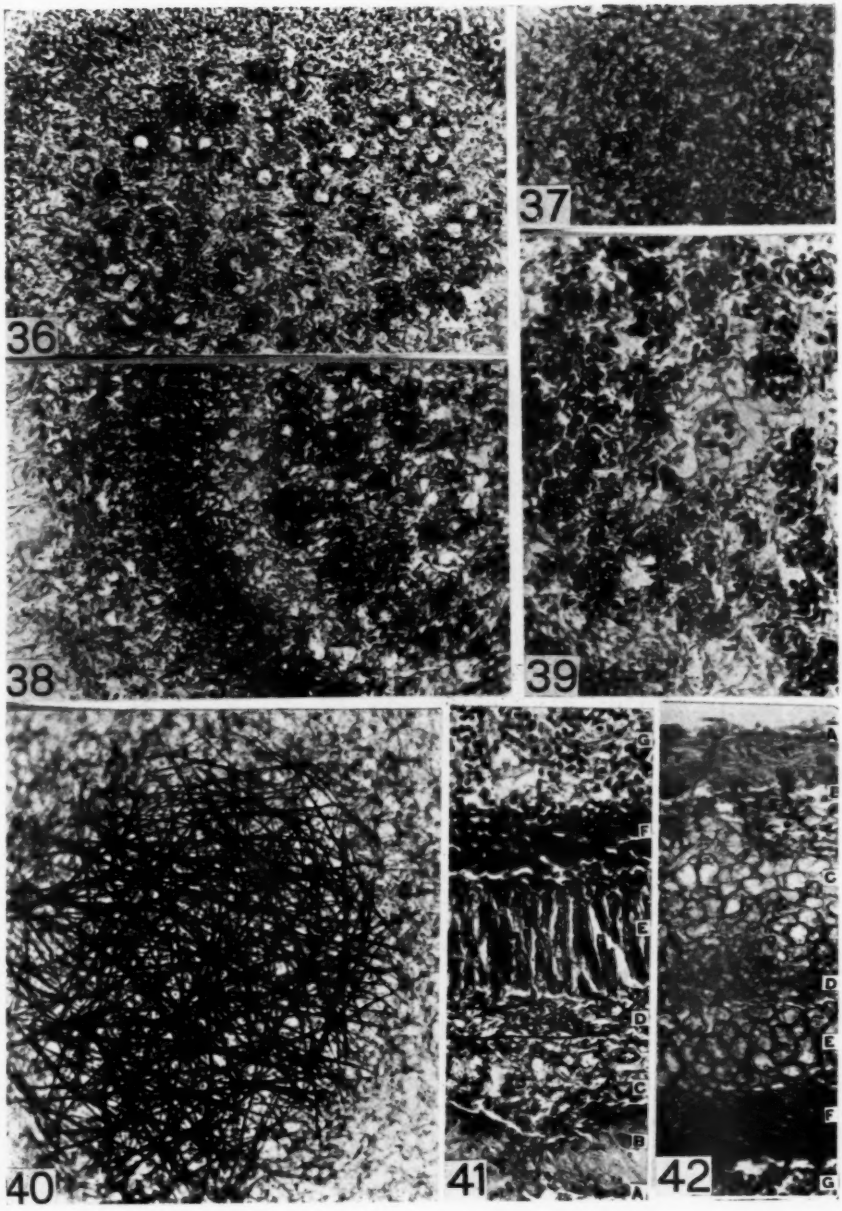

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PLATE 21

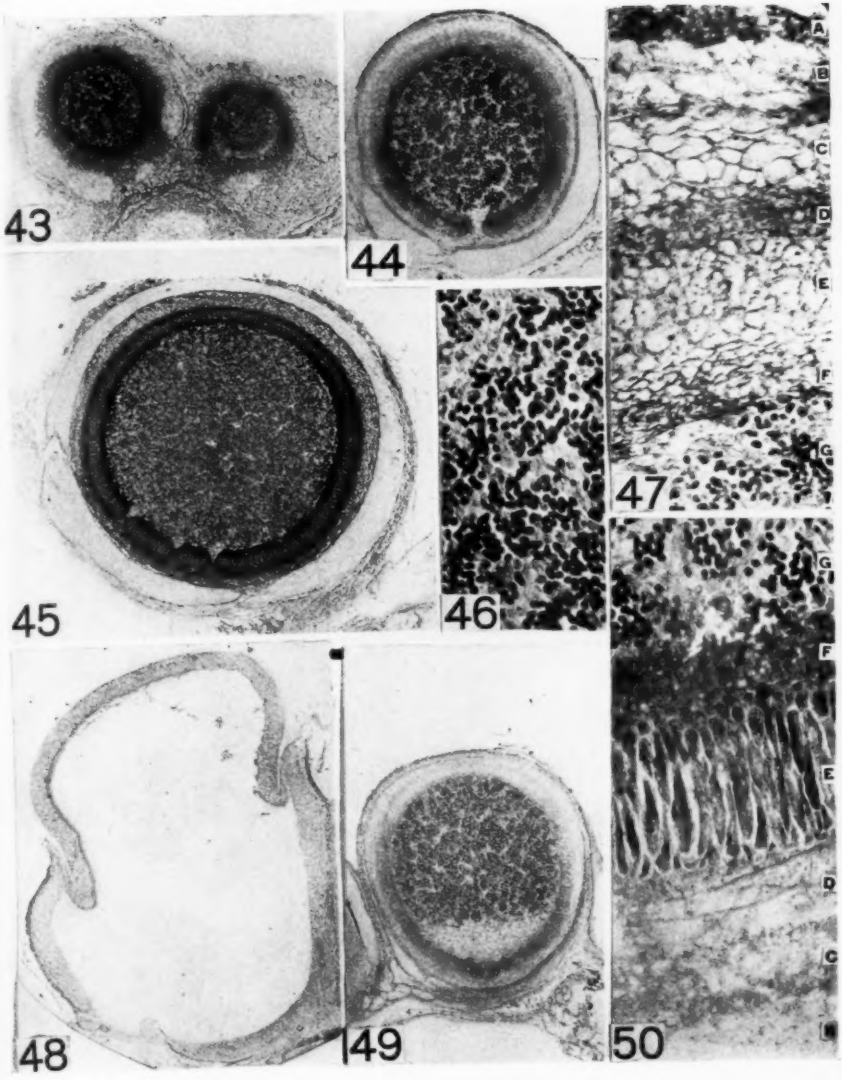

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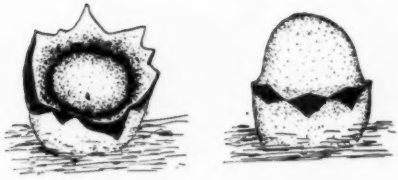
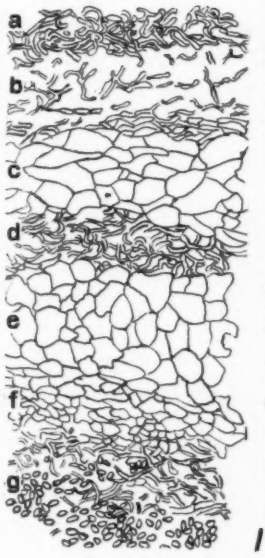
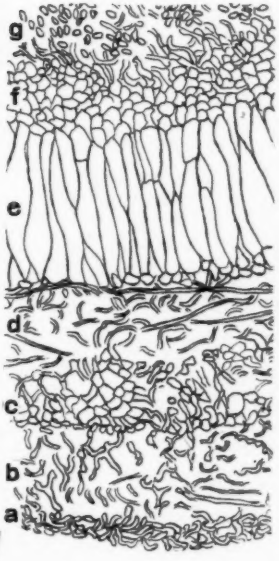
PLATE 22







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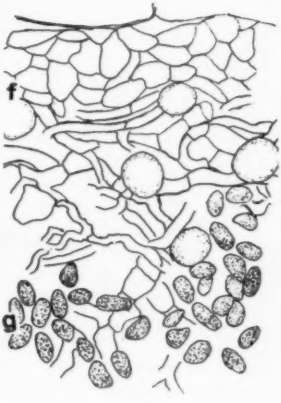

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PLATE 23

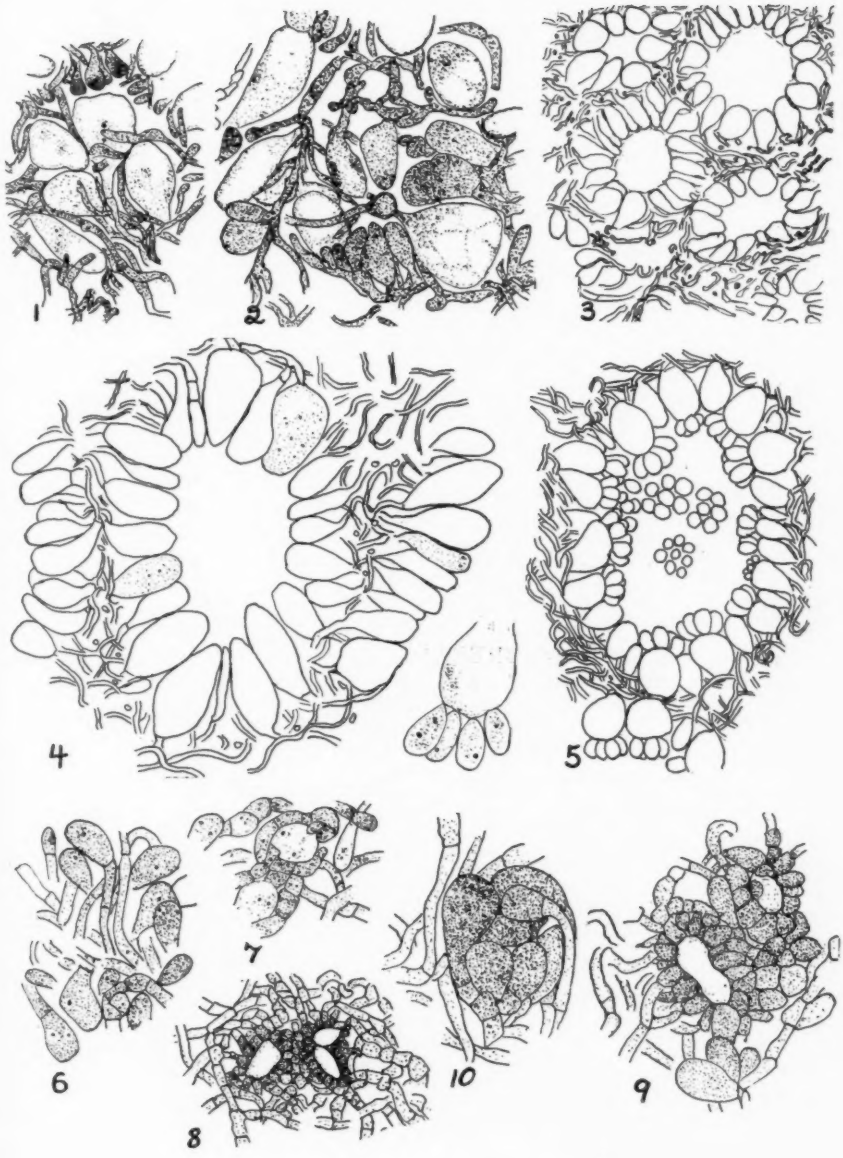

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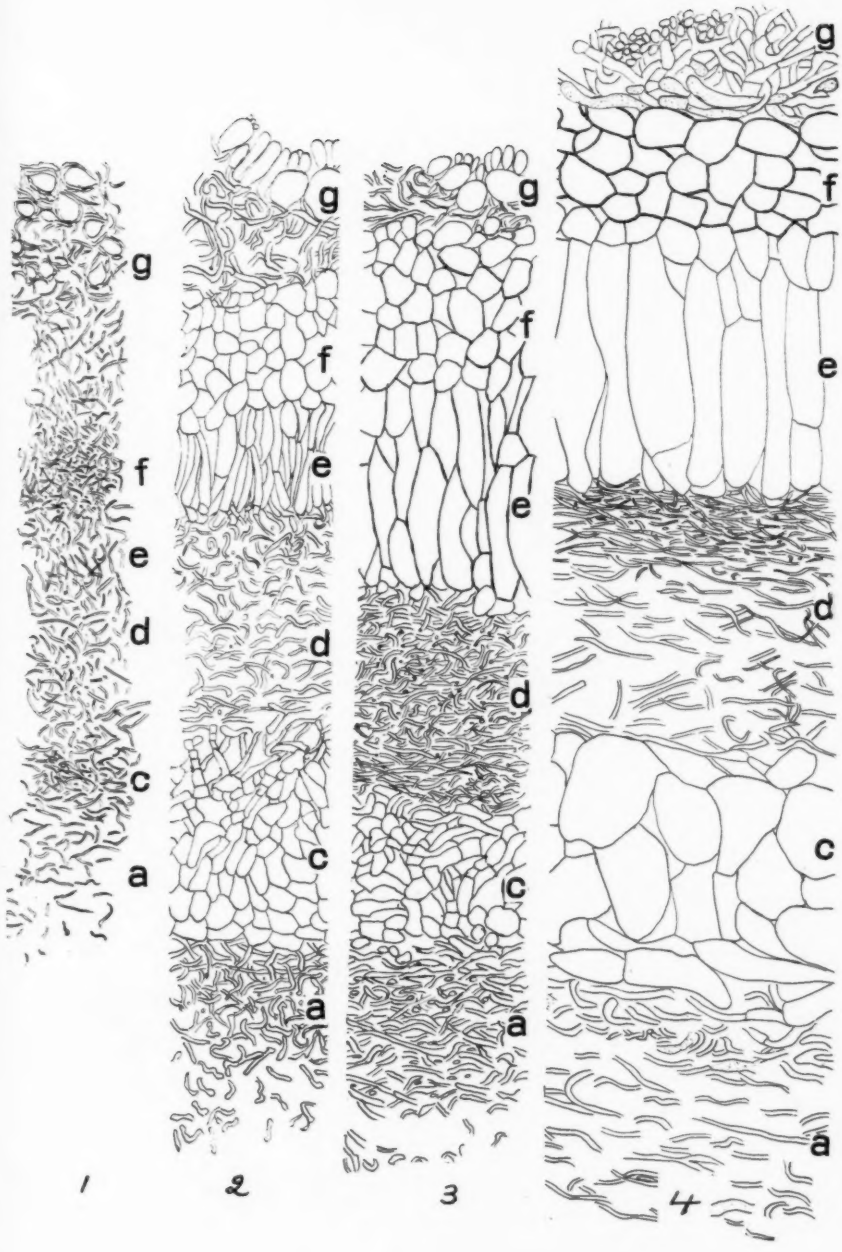
PLATE 24



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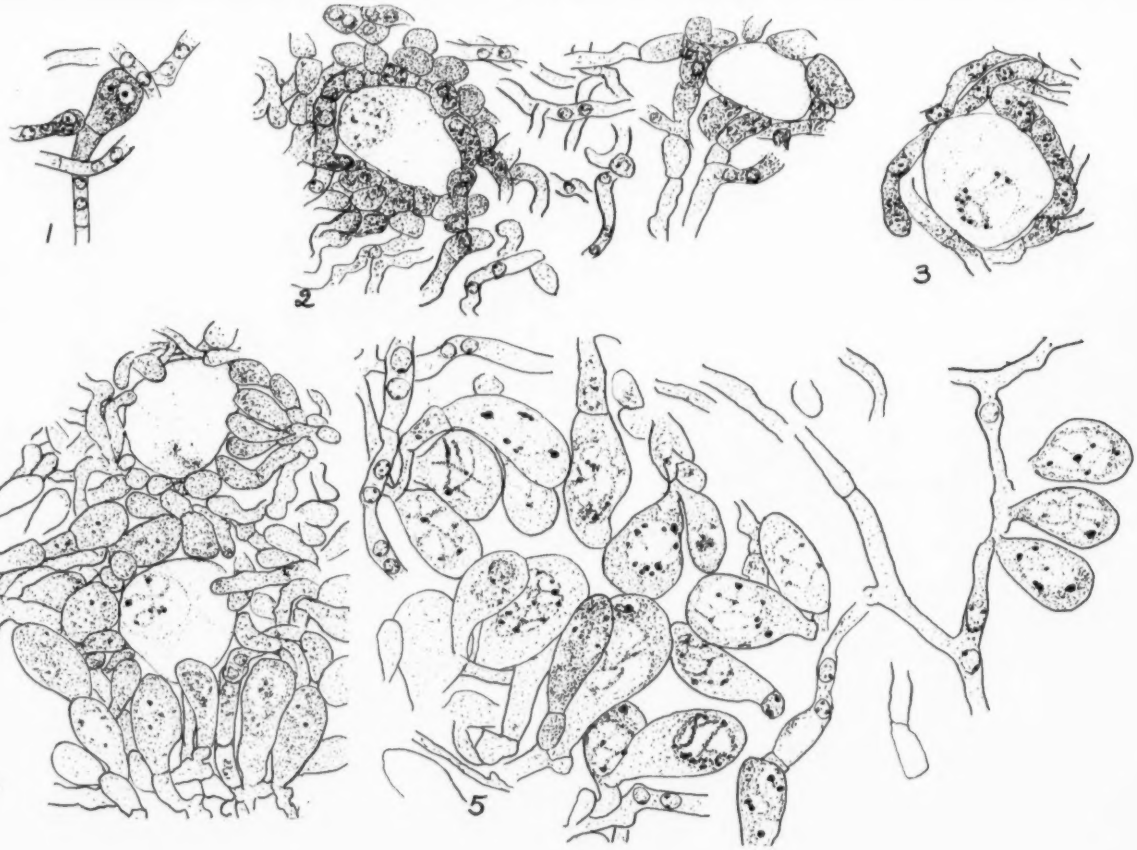
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THE AGE OF THE UNIVERSE

By J. H. Swartz

Every star is radiating energy into space at a tremendous rate. In the case of our sun, the heat radiated at the sun's surface per square metre per minute is nearly 1,000,000 calories. If the sun were made of solid coal, the burning of which furnished the heat, it would not last 5,000 years. Or, to put it another way, since the sun is 332,000 times as heavy as the earth, it would take 66 earths of coal per year to furnish the sun's heat. The source of this tremendous stellar energy has long been a matter of profound interest and much conjecture.

Helmholtz suggested that the source of the sun's heat might be its contraction and showed that a contraction of 200 feet per year would be sufficient to account for the entire annual output of the sun. This would mean that the sun some 12,000,000 years ago completely filled the earth's orbit, so that the earth could not be over 12,000,000 years old. Yet the minimum age of the earth is shown by geological methods to be not less than 100,000,000 years, and our most reliable method indicates ages of a billion and a half for our oldest rocks and perhaps eight billion years for the earth's crust. Furthermore observations on the changing period of the cepheid variable δ-Cephei indicate a rate of contraction only about 1/800th of that demanded by the contraction theory. Contraction is thus obviously entirely inadequate.

Radioactivity has been suggested as a possible source of heat. Moulton has shown that even if the sun were composed entirely of Uranium it would yield insufficient heat to account for the known age of the earth.

Chemical changes have also been offered as a possible source of stellar energy. The energy available from this source is negligible, however. It is estimated by Jeffreys[1] to be on the order of 10^{11} ergs per gram as compared with 10^{16} ergs per gram for contraction and 10^{21} ergs per gram for mass conversion (*q. v.*).

[1] Jeffreys, H. The Earth, p. 72. Cambr. Univ. Press, 1924.

Einstein has shown that mass is theoretically convertible into tremendous quantities of energy. His equation is

$$E = \Delta M \cdot c^2 \qquad (1)$$

where E is the energy formed (in ergs), ΔM is the mass converted (in grams), and c is the velocity of light (2.9986×10^{10} cm./sec.).

The conversion of one gram of matter into energy would furnish as much heat as approximately 30,000 tons of coal. It was Jeans[2] who first suggested that this conversion of matter into energy might be an

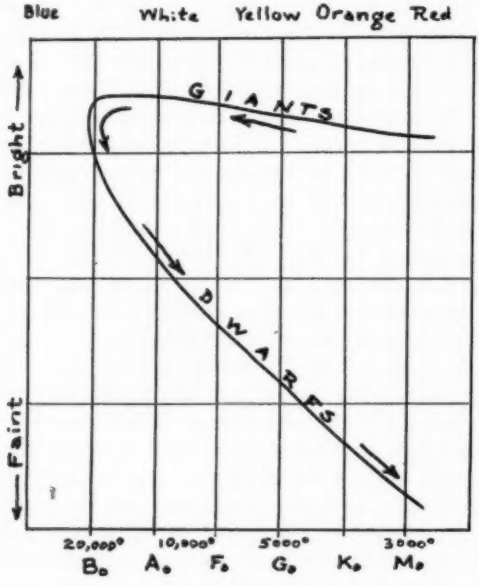

FIG. 1

important source of stellar energy. He has been followed by Eddington, Russell, Jeffreys, and many others.

Hertzsprung and Russell have shown that the stars fall naturally into two groups: (1) a group of Giant stars whose brightness is independent of temperature, and (2) a group of Dwarf stars whose brightness decreases with decreasing temperature. Russell[3] has advanced a very plausible and widely accepted theory of stellar evolution in which

[2] Nature **70**: 101. 1904.
[3] Nature **93**: 227–230; 252–258; 281–286. 1914.

all stars begin as cool, highly distended giants. They lose heat and contract, whereupon their temperature rises, in accordance with Lane's paradoxical law. Thus each unit of their surface gets brighter as they get smaller so that their brightness remains approximately constant. Finally a density is reached where the ideal gas laws and Lane's law break down. Further cooling brings not only a decrease in size but also a decrease in temperature with a corresponding decrease in surface brightness (brightness per unit area) and total brightness, which causes the star to turn and pass down the dwarf series, as shown in figure 1.

TABLE 1

UNITS: TEMPERATURE, 1,000°C. ABS.; MASS, MASS OF SUN; DENSITY, G./CC.

SPECTRAL CLASS	EFFECTIVE TEMPERATURE—GIANTS*					MEAN MASS		MEAN DENSITY	
	Abbot	Potsdam	Coblentz	Saha	Fowler and Milne	Giants	Dwarfs	Giants	Dwarfs
O_a		23		23					
O_5					30	50			
B_0		20	13	18	19	10		0.04	
B_3					16	9		0.22	
B_5						8.3		0.20	
B_8	16					7.3		0.24	
A_0	14	11	8	12	10	7.0	6.0	0.16	0.36
A_5		9				5.6	4.0	0.071	0.40
F_0		7.5		9	7.5	4.3	2.5	0.025	0.40
F_5	6	7.2	6			3.2	1.5	0.0078	0.39
G_0	5.8	6.5	6	7	6	2.6	1.0	0.0025	0.68
G_5		4.5				2.8	0.76	0.00087	1.2
K_0		3.7	4		4.5	3.0	0.68	0.00018	1.3
K_5	3	3.5	3.5		3.9	2.6	0.62	0.000026	1.4
M_0		3	3	5	3	2.0	0.59	0.0000096	5.4
M_5	2.5	2.95		4					
N		2.3							

* Temperatures of Dwarfs are 10% to 20% higher than Giants of the same class (indirect methods).

Each temperature is characterised by certain color and spectral characteristics, so that both Giants and Dwarfs pass through a definite and continuous sequence of spectral, color, and temperature changes. These spectral changes fall naturally into some twelve divisions or "spectral classes," as they are called, which have been designated by certain arbitrarily chosen letters. The six main classes, which comprise 99% of all the stars, are known, in order of descending temperature, as classes B, A, F, G, K, and M. As in any cooling object, the color at the same time passes from bluish-white through white, yellow, orange, to red, as

indicated in Fig. 1. The changes are so perfectly continuous that it has been found possible to divide each class into ten successive steps or gradations, designated B_0, B_1, B_2, . . . B_9, A_0, A_1, etc.

If we leave the Giant stars and concentrate our attention on the Dwarfs, we find that the Dwarfs decrease not only in temperature, brightness, and color, but also *in mass*. Joy[4] and Seares[5] give the following temperatures, mean masses, and mean densities for the different spectral classes.

The decreasing masses of the several spectral classes may be due to several different factors. (a) Part of the difference may be accounted for by the way in which the stars used for the measurements were selected, although Seares[6] removed this difference as far as possible. (b) Part of the difference may be due to original differences in mass, the smaller dwarfs, as shown by Russell,[7] tending to predominate in the later spectral classes. (c) Or part of the difference, and this seems very probable, may be due to the conversion of some of the mass into energy, as postulated above.

Positive evidence seems now to be added to the theoretical considerations advanced above by the recently discovered Millikan rays,[8] which demand potentials on the order of 32,000,000 volts for their production. Jeans[9] and Eddington regard the conversion of matter into energy as the only probable source of such energy, although Millikan[10] himself does not entirely concur in this view.

There is thus a very considerable basis for the assumption that the stars receive much of their energy from the conversion of matter into energy. Furthermore, this latter source of energy is so great that all others (contraction, chemical change, etc.) are negligible in comparison.

Age of the "Universe." Let H_F be the total energy set free within a star during time t years, and let H_R be the average energy radiated per year during that period. We then get

$$t = \frac{H_F}{H_R} \qquad (2)$$

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[4] Int. Crit. Tables, Vol. **1**, p. 385. McGraw-Hill, 1926.

[5] Astrophysical Journal **55**: 179, 202. 1922.

[6] *Loc. cit.*, pp. 179–182.

[7] *Loc. cit.*, p. 285.

[8] Science, Nov. 20, 1925; Phys. Rev. **27**: 353–361, 645–658. 1926; **28**: 859–868. 1926.

[9] Nature **116**: 861. 1925; Nature **117**: 18. 1926.

[10] Phys. Rev. **28**: 867–868. 1926.

In order to determine not only the total age of the star, but also, what is even more interesting, the length of time required for the star to pass through the different stages of its evolution, we shall divide its life history into Giant and Dwarf sequences, and further subdivide these into stages of half a spectral class, i.e. B_0-B_5, B_5-A_0, etc. Within such small stages the actual values of H_F and H_R depart but little from their average values for the stage, and a solution well within the limits of accuracy of the available stellar data may be obtained without the use of integral calculus.

In our solution we will disregard the Giant sequence entirely, for two reasons: (1) the data for the Giant stars is not as clear as that for the Dwarfs, and (2) the star passes so rapidly through its giant stages and so slowly through its dwarf stages that the time spent as a Giant is negligible.

Evaluation of H_F. As shown in table 1 above, there is a continuous decrease in mass in passing down the Dwarf series. We have already seen that there is considerable reason to regard much of this loss as due to the conversion of matter into energy. Assuming the total mass loss (ΔM) to be due to such annihilation of matter, we may calculate, by means of Einstein's formula [Equ. (1), above], the amount of energy set free during each stage through which the star passes.[11]

We have seen above that all other factors are negligible in comparison with mass conversion, so that our formula for H_F becomes

$$H_F = \Delta M \cdot M_\odot \cdot c^2 \tag{3}$$

where ΔM, since it is given in tables 1 and 2 in terms of the mass of the Sun, must be multiplied by $M_\odot$, the mass of the Sun in grams (1.9×10^{33} g.), to be converted into grams as demanded by equation (1) above.

Evaluation of H_R. On the basis of the usual assumption that the stars radiate as black bodies, we get, from Stefan's law,

$$H_R = K\sigma AT^4 \tag{4}$$

where K is the number of seconds in one year (31.5569×10^6), σ is Stefan's constant (5.709×10^{-5}), A is the area of the star's surface, and T is its absolute temperature.

[11] Since some of the decrease in mass is certainly due to other factors than mass conversion, we will get, on this assumption, maximum values of H_F and t.

Evaluation of A. In equation (4) A is unknown but can be determined from the values of M (mean mass) and ρ (mean density) given in table 1; for, if R = the radius of the star, and $M_\odot$ = the mass of the Sun in grams, we get,

$$\text{Volume} = \frac{4}{3}\pi R^3 = \frac{M \cdot M_\odot}{\rho}$$

from which

$$R = \left(\frac{3}{4\pi}\right)^{1/3} \left(\frac{M}{\rho}\right)^{1/3} (M_\odot)^{1/3} \tag{5}$$

Therefore,

$$A = 4\pi R^2 = (36\pi)^{1/3} (M_\odot)^{2/3} \left(\frac{M}{\rho}\right)^{2/3} \tag{6}$$

and

$$H_R = K\sigma(36\pi)^{1/3} (M_\odot)^{2/3} \left(\frac{M}{\rho}\right)^{2/3} T^4. \tag{7}$$

Value of t. Substituting equations (3) and (7) in equation (2), we get

$$t = \frac{M_\odot \cdot c^2}{K\sigma(36\pi)^{1/3} (M_\odot)^{2/3}} \cdot \frac{\Delta M \cdot \rho^{2/3}}{M^{2/3} T^4}$$

or, giving the constants of the first term their proper values,

$$t = 1.2783 \times 10^{23} \frac{\Delta M \cdot \rho^{2/3}}{M^{2/3} T^4} \tag{8}$$

Results. The values of T for Giant stars given in table 1 were plotted and a freehand curve drawn through them, as shown in figure 2. Since Dwarf temperatures are from 10% to 20% higher than Giant temperatures, a second curve (see fig. 2) was drawn which was 20% higher for M_5 stars, gradually approached the first curve with earlier and earlier spectral class, and finally merged with it for B_0 stars. The dwarf temperatures given in table 2 below were read from this second curve. The other data for table 2 were obtained from table 1.

The values of *t*, in trillions of years, derived from equation (8) are given in the last column of the table. It is not likely that the apparent decrease in age of the G_5-K_0 stage is real. Such a decrease would be difficult to account for in view of the subsequent continued increase.

It is probable that the Giant sequence is not much more than ten times as long as the $B_0 - B_5$ stage, or a total of 0.145 trillion years,

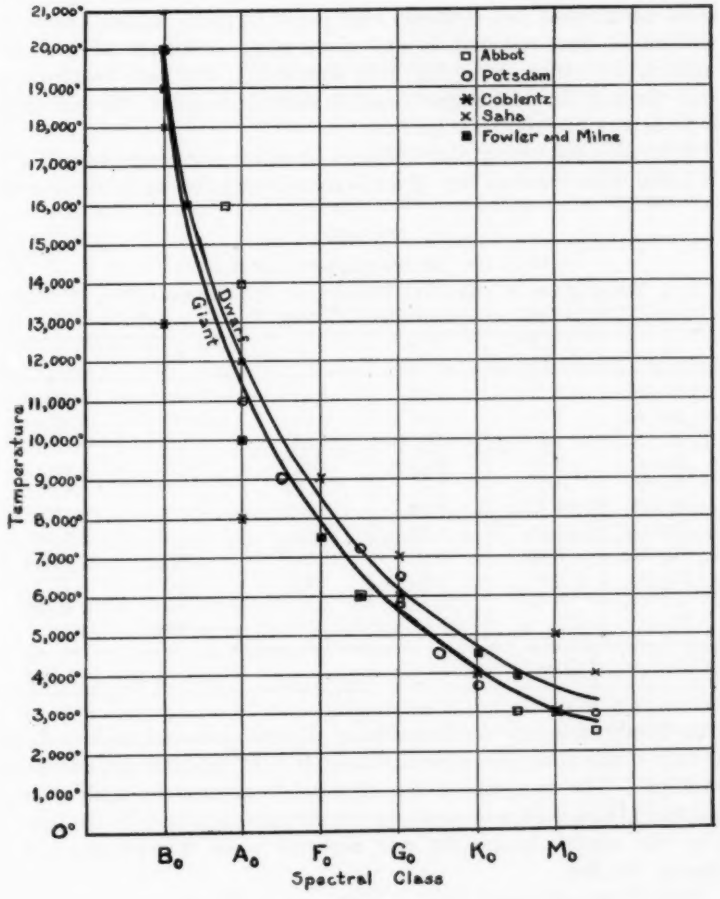


FIG. 2

which makes the total age of the entire Giant and Dwarf sequence, from M_0 Giant to M_0 Dwarf, only about 17.78 trillion years.

It is difficult to know what the history of the star is beyond M_5 or

what time is involved in its evolution beyond M_0. It is unsafe to extrapolate the time curve beyond M_0, since it seems highly improbable, in view of the greatly decreased mass conversion of that stage, that mass annihilation can continue very much further. Unless the total amount of heat radiated in any given stage continues to decrease more rapidly than the dying mass conversion, the time required to pass through the final stages would be shortened again. All this, however, is pure speculation at present.

Relation to the Binary Star Problem. A rather interesting coincidence is found when the data here given is considered in reference to the cap-

TABLE 2

DATA FOR AND SOLUTION OF EQUATION (8)

UNITS: MASS, MASS OF THE SUN; DENSITY, G./CC.; TEMPERATURE, °C. ABS.; TIME, 10^{12} YEARS

STAGE	ΔM	M	ρ	T	t
B_0-B_5	1.7	9.15	0.12	17,000°	0.01447
B_5-A_0	2.3	7.15	0.28	13,500°	0.10208
A_0-A_5	2.0	5.0	0.38	10,975°	0.31617
A_5-F_0	1.5	3.25	0.40	9,225°	0.65511
F_0-F_5	1.0	2.0	0.395	7,875°	1.12722
F_5-G_0	0.5	1.25	0.535	6,750°	1.74857
G_0-G_5	0.24	0.88	0.94	5,840°	2.75607
G_5-K_0	0.08	0.72	1.25	5,065°	2.24453
K_0-K_5	0.06	0.65	1.35	4,420°	3.27123
K_5-M_0	0.03	0.605	3.4	3,870°	5.40403
M_0-M_5				3,450°	
Total Age of Dwarf Sequence					17.63948

ture theory of binary star formation. There is a considerable body of evidence indicating that approximately 40% of the stars are double or multiple. The tremendous time required to account for such a large number of binaries has always been the strongest objection to the theory that the second component was captured when it approached, too closely, the first.

Jeans[12] has estimated that, if there are a billion stars arranged in cubic piling within a space corresponding to a sphere of 3,260 light years radius, any given star "is only likely to experience a nontransitory

[12] Mem. R. A. S. **72**, pt. I, 1917.

encounter about once in 4×10^{13} years." In other words, in 40 trillion years 100% of the stars would have experienced non-transitory encounters which might have led to the formation of double stars. In the 17.78 trillion years of our calculations we could expect that $\frac{17.78}{40}$ or 44.5% of the stars would have experienced such encounters, a figure which agrees remarkably with the percentage indicated by modern studies. There are, however, a good many objections still to be faced by the capture theory: the high percentage of A-type binaries and the lack of any constant increase in numbers among later type stars, to mention only a few. The coincidence is probably, therefore, not too significant.

SUMMARY

1. It is probable that much of the energy of the stars is derived from the annihilation of matter and its conversion into energy.

2. Assuming this to be true, it is shown that the age of a star may be calculated from the formula

$$t = 1.2783 \times 10^{23} \frac{\Delta M \cdot \rho^{2/3}}{M^{2/3} T^4} \text{ years} \qquad (8)$$

in which ΔM is the mass converted into energy during time t, and M, ρ, and T are the mean mass, density, and absolute temperature of the star during that period.

3. Present stellar data indicates a maximum age of something over 17.78 trillion years for our present stellar universe.

4. This gives a percentage of stars which would have approached closely enough to permit capture and the formation thereby of a double star, of 44.5%—in striking, though probably insignificant, coincidence with the approximately 40% of binaries indicated by present data.

CHAPEL HILL, N. C.

THE INCUBATION OF HEN EGGS UNDER INCREASED ATMOSPHERIC PRESSURE

By Bert Cunningham

Plate 26

There are several instances reported of attempts to incubate various kinds of eggs under reduced pressure. A number of experiments have been carried on with increased pressure, but so far as the writer knows, none have been carried out with hen eggs.

The idea of the experiment originated in the discovery that considerable pressure develops in the eggs of *Chrysemys cinerea*. The writer thought that there might be some relation between this and the slow rate of development of the turtle. Plans for the work were made and preliminary experiments run some three years ago. These were so contrary to expectation that it was desirable to run the experiments under better control.

During last summer series of experiments were begun with better checking apparatus. The results of the experiments are to be set forth in this paper.

Apparatus

The apparatus consists of a pressure cooker, an egg carrier, a reserve tank for compressed air, an air compressor, a pressure control valve, and a large electrical incubator and a thermograph. It is desirable to add a recording barometer.

In the earlier experiments where a hand automobile pump was used, it was impossible with the apparatus in use to keep the pressure anywhere near constant. An automatic pressure pump has now been installed and more constant pressure can be maintained.

In the earlier experiments wooden egg carriers were used but these gave off odors which might or might not have some effect on the embryo. They were later replaced by metal carriers.

Considerable difficulty has been experienced in some clutches on account of molds. This problem is being attacked. It appears, although by no means certain, that mold grows alike on shells containing either

dead or live embryos. It also appears, though not conclusively proved, that gasoline fumes in very dilute form will prevent this growth in either live or dead embryos. The pressure reservoirs have both been used for gasoline. When, for a short period of time, these tanks were out of service and the air was delivered directly to the cooker by a hand pump, mold developed in all cases. Although many clutches have been run in air from these pressure tanks, in only one case has considerable mold developed; in this case the embryos had been dead for a considerable time when opened.

CHECKING THE APPARATUS

The pressure cooker containing the eggs and a moist towel was placed in the incubator, the thermograph was placed alongside, and check eggs in ordinary egg cases were placed alongside the thermograph. Check eggs, therefore, were secured in most cases. The temperature inside the cooker was checked with that outside by periodic readings of the thermometers. It seemed to correspond well. It would be well to have a week's thermograph record taken simultaneously inside and outside the cooker.

THE EGGS

The eggs for these experiments were all secured from the same flock of Rhode Island Reds. The poultry man who furnished them was cautioned to bring none except such eggs as were collected the day they were laid. Even under the most careful collection it is to be remembered that eggs are laid in considerably different stages of development. For that reason larger numbers of eggs have been used than would have been used otherwise. A cold spring, or some other cause, gave a considerable percentage of infertile eggs in some clutches. This and the smallness of our pressure cooker have spread the experiment over a considerable period of time.

THE EXPERIMENTS

The experiments consisted of increasing the pressure in the cooker until it reached from 15 to 18 pounds, then incubating at 37½ degrees. Previous experiments in these laboratories indicated that this was a more favorable temperature for all purposes than 39½ degrees. As already indicated, in earlier experiments the pressure would drop over night to approximately 5–8 pounds. For this reason, although the

results in the preliminary experiments were similar to those secured later, these earlier chicks are not described here.

With improved apparatus and more care the pressure has been carried between 12–20 pounds during the 24 hour periods. More precise apparatus now being built will keep even more constant pressure. Under these later conditions approximately 36 eggs have been incubated. Of these 6 or 8 were sterile, 5 produced considerably abnormal embryos, and 15 have produced apparently normal chicks, while the remaining were destroyed by mold. One chick seemed to be abnormal only in respect to red blood cells. Chicks incubated under these conditions were consistently more advanced than those under normal pressure run as checks, as may be seen by the accompanying photographs.

In the earlier experiments chicks were taken about every day; thus a fairly good series of chicks was secured. Among these occurred the chick deficient in red blood cells mentioned above. Since this chick had been incubated for five days under pressure it was desirable that it be compared with normal pressure five-day chicks and increased pressure five-day chicks. Therefore, an embryological examination is being carried out on the five-day chicks and comparisons will be given in a later paper.

The oldest chick secured alive under pressure incubation conditions was 17½ days. In this chick all the yolk materials had been absorbed, and the chick was apparently nearly ready for hatching.

An examination of the specimens secured shows that the greatest advantage in growth seems to lie in the first few days of development. In the one case of its kind that has been taken, the application of pressure for five days and then return to normal pressure has given a little more advanced condition than would have been reached under continued pressure conditions. Both cases are far in advance of the chicks secured under normal pressure, as may be seen in the accompanying photographs.

Observations show that chicks can survive a greater fall of temperature under increased pressure than they can under normal pressure. Other observations indicate that they are more easily killed at temperatures above 103½ degrees under increased pressure than under normal pressure.

Further studies are to be made of the relation of pressure and temperature, the determination of the lethal pressure at various temperatures, the lethal temperatures at various pressures, and the effects of changing the partial pressures of the gases of compressed air. There

are also many other problems which are suggested by the results already attained.

The results show that incubation of hen eggs can be carried on under increased pressure up to two atmospheres without any serious difficulties, and that the embryos produced under these conditions are advanced over normal pressure chicks. Although no chicks have actually hatched under these conditions, it is reasonable to suppose that they may be, since chicks have been carried for 17½ days and removed alive.

DUKE UNIVERSITY,
DURHAM, N. C.

EXPLANATION OF FIGURES

(All specimens shown here were alive when removed from egg)

Fig. 1. A. Embryos from eggs incubated under normal conditions.
B. Embryos from eggs incubated under 8–15 pounds pressure. Both sets incubated simultaneously in the same incubator.

Fig. 2. A. Seven day chick under normal pressure. Check chick for B.
B. Seven day chick under 15–18 pounds pressure. Simultaneously incubated in same incubator with A.

Fig. 3. A. Largest and smallest embryos from eggs incubated at from 8–15 pounds.
B. Largest and smallest embryos from eggs incubated at normal pressure. A and B incubated simultaneously in same incubator.

Fig. 4. A. Eleven day chick, pressure normal.
B. Eleven day chick, pressure 15–18 pounds.

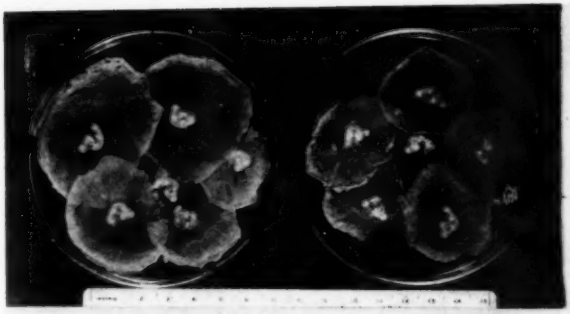
Fig. 5. A. Twelve day chick, pressure normal.
B. Twelve day chick, pressure 15–18 pounds.

Fig. 6. A. Fourteen day chick, pressure normal.
B. Fourteen and one-half (approximately) day chick, pressure 15–18 pounds.

Fig. 7. B. Same chick as 6B.
C. Fifteen day chick, first five days incubation under 15–18 pounds, next ten at normal pressure.

Fig. 8. Seventeen and one-half day chick incubated under pressure. Practically all yolk was absorbed.

PLATE 26



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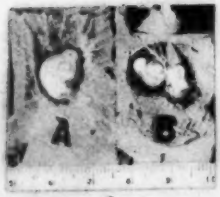

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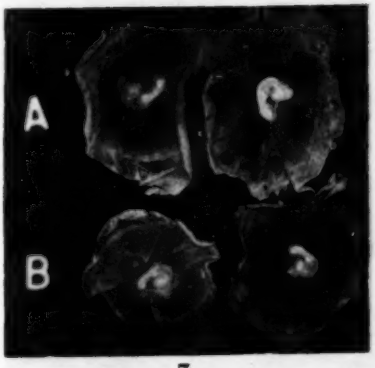

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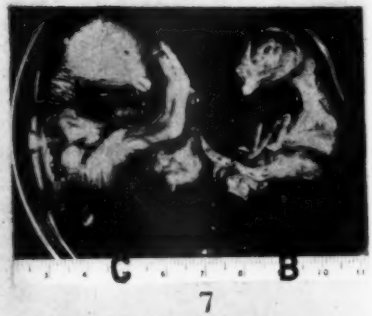

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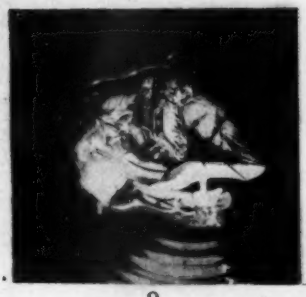

8

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TRILLIUM IN NORTH AND SOUTH CAROLINA

A Critical Systematic Reconnaissance

By Donald Culross Peattie

The genus *Trillium* L., common in temperate North America except the Great Plains and the Southwest, and sparingly known in Japan, China, and Siberia, reaches its greatest development in the southeastern United States. In North and South Carolina alone are to be found about 13 species and in the mountainous portions of these states the genus is individually and specifically numerous, forming as it does some of the showiest aspects of the spring flora.

In the field the number of species, varieties and forms which really exist is large enough, but in the herbarium and library there are so bewildering a lot of forms hastily described, and so much synonymy, and so many poor specimens, that there are few plants harder to name correctly than a *Trillium*. The identification of herbarium material is unsatisfactory by reason of the fading of the colors in drying, the mashing of the soft petals, and the paucity of collector's field notes.

The treatment of *Trillium* in Small's *Flora of the Southeastern U. S.* (ed. 1 & 2) is practically adequate for the sessile-flowered group, but for the much larger, pedunculate series, it is incomplete; that in the same author's forthcoming revised work on the same region is to be a distinct improvement, but it recognizes none of the finer gradations of classification which are needed to account for a genus that will not be sharply delimited as to species.

Linnaeus knew only three species of *Trillium*, but Rafinesque described a host of new species which unfortunately are so sketchily characterized that it is difficult to know what he meant. Following Asa Gray, who eschewed anything from Rafinesque's pen, Sereno Watson in Proc. Am. Acad. Sci. XIV (1879) reduced to synonymy or varietal rank the greater part of Rafinesque's and other writers' names of *Trillium*. It was not until Small showed the limited nature of *T. sessile* L. and set off *T. Underwoodii* and later *T. Hugeri* that our knowledge of the genus drifted out of the doldrums into which workers like Watson, with almost no field knowledge, had put us.

Gleason's monograph of the pedunculate series, and Gates' descriptions of new species, added largely to our knowledge of the more complex group of *Trillium*, and it might be thought that all one would need to do would be to compare and compile.

But in the case of *Trillium* there are problematic aspects to systematic study. In the first place there is little doubt that within species there are strongly marked races, color and size variants and sports given to exhibiting themselves in showy forms. These are likely to occur in any species of *Trillium* and in almost any large patch of *Trillium* plants. Many of the variations appear to be self-perpetuating and, crossed with other eccentric or normal members of the species, result in hybrids where the parental characters are exhibited in blend or in mosaic.

In a genus where hybrids are common and where the species are without economic importance, any tendency to give definite names to every minutely different phase of growth is unscientific.[1]

In addition to the variants of color and size there is another difficulty in the path of systematic botany—the matter of teratology. About fifty notes and articles have been written on the abnormalities of this most aberrant genus. Calyces and corollas turned to leaves, stamens turned to petals, complete or partial viridism or albinism of any of the floral organs, all or part of the floral and leaf parts in fours or in a far more complicated phyllotaxy, leafless plants—all of these and many others have been repeatedly noticed, especially in *T. grandiflorum*, *T. erectum* and *T. sessile*.

It is a temptation to give some sort of Latin name to all these abnormalities, and this temptation has been yielded to regrettably. While we cannot have too much evidence on such important phenomena as those which show the common origin and homology of leaves and floral organs, still, the tendency to give these monstrosities standing in systematic botany is a bad precedent.

Lastly, the lengths of floral organs have often proved unstable characters, as Harbison has shown in his measurements of growing flowers (Bilt. Bot. Stud. **1**: 24. 1901). In *T. ludovicianum* the length of the

[1] This is not to say that every difference and aberration might not be carefully noted and even classified in some way by anyone interested in genetics, color inheritance, mutation, or ornamental gardening. Particularly interesting results might be obtained by an attempt to study *Trillium* hybrids, and experimental work might be conducted. There seems to be reason to think that some hybrids follow Mendelian lines (Gates; Ann. Mo. Bot. Gard. **4**: 46. 1917).

petals doubled in 10 days, but the width only increased by 50%. The length of the sepals increased only about 55% and the width about 40%; the length of the filaments and anthers on the other hand increased scarcely at all, the length of the stigmas 40%. Thus such varieties as *T. luteum* var. *latipetalum* Gates, based on a single herbarium specimen, represents perhaps only a plant caught at a single instant of its growth. A few days later it might have become a different variety!

A proper discussion or study of *Trillium*, then, should bear in mind the following points:

1. The unusual necessity for a good field knowledge and the untrustworthiness of herbarium specimens.
2. The making of full notes, not only on abnormalities, but notes on color, size and habit to accompany normal specimens.
3. The invalidity of hybrids, monstrosities, and gigantism and dwarfing in a classification of pure species.

I have concluded to give the rank of form (*forma*) to all simple or practically simple color variants, such as albinism and viridism; but white stripes upon sepals, etc., I have regarded as monstrosities. What constitutes a monstrosity it may be difficult to ascertain but under this heading I think multiplication or lack of any organs, or partial or complete transformations of any organs to resemblances to others, should be so classified.

The following account hardly aims to be a fresh contribution to systematic botany, but is merely a preliminary clearing of the way for more extended observations by myself and, I hope, by others. I have limited my scope to North and South Carolina because only there can I furnish observations upon the living plants.

KEY

A. Flower sessile
  B. Petals sessile, not greenish except in *T. discolor*
    C. Anthers less than 1 cm. long; petals broadly spatulate, greenish or pale yellow — *T. discolor* 1.
    C. Anthers 1 cm. long or more; petals not broadly spatulate, rarely greenish, mostly narrow, brown, purple, or yellow
      D. Leaves seldom more than 8 cm. long; sepals about 2–3.5 cm. long
        Petals maroon or purple or brownish — *T. sessile* 2.
        Petals yellow or greenish — *T. sessile* f. *luteum*
      D. Leaves seldom less than 10 cm. long; sepals about 4–5.5 cm. long

E. Leaves about as broad as long; filaments more than 2 mm. long
Petals maroon or brown — *T. Hugeri* 4.
Petals yellow or greenish — *T. Hugeri* f. *flava*
E. Leaves distinctly longer than broad; filaments less than 2 mm. long — *T. Underwoodii* 3.
B. Petals clawed, green — *T. viride* 5.
A. Flower peduncled (rarely sessile in *T. pusillum*)
F. Ovary 6-angled; leaves sessile except in *T. Catesbaei* and abnormally in *T. grandiflorum*
G. Stigmas free, sessile on the ovary, that is, style none
H. Stigmas more or less curled
J. Ovary dark purple or nearly black; petals not recurved
K. Stamens not surpassing the stigmas; flowers often ill-scented
Petals claret red — *T. erectum* 7.
Petals greenish — *T. erectum* f. *viridiflorum*
Petals white or white striped with purple — *T. erectum* var. *album*
K. Stamens distinctly longer than the stigmas; flowers often sweet-scented
L. Filaments 8–12 mm. long, approximately equalling the anthers which have purple connectives; leaves broadly ovate, long-acuminate
Flowers red — *T. Vaseyi* 8.
Flowers white — *T. Vaseyi* f. *album*
L. Filaments 2–6 mm. long, distinctly shorter than the yellow anthers; leaves broadly rhombic, short-acuminate — *T. simile* 9.
J. Ovary white or rarely pink
Petals recurved, pedicel always declined below the leaves — *T. cernuum* 10.
Petals erect or merely spreading; pedicel erect or merely horizontal — *T. erectum* var. *album*
H. Stigmas practically straight
Petals large, cyclically disposed
Flowers white (sometimes fading rose) — *T. grandiflorum* 12.
Flowers rose — *T. grandiflorum* f. *roseum*
Flowers green — *T. grandiflorum* f. *viride*
Petals small, spirally overlapping — *T. grandiflorum* var. *parvum*
G. Stigmas united below into a short style
Leaves narrowly elliptic; peduncle erect (flower rarely sessile) stamens straight; filaments about ½ as long as the anthers — *T. pusillum* 6.
Leaves broadly elliptic; peduncle nodding; stamens curved; filaments fully as long as the anthers — *T. Catesbaei* 11.
F. Ovary 3-lobed; leaves petioled — *T. undulatum* 13.

1. **T. discolor** Wray in litt. Hook. Bot. Mag. pl. 3097. 1831.
T. sessile var. *Wrayi* Wats. Proc. Am. Acad. **14:** 273. 1879.

Stems smooth, 1–3 dm. tall; leaves oval, 4–7 (rarely up to 16) cm. long, acuminate, mottled; sepals 2 cm. long, spreading; petals about 1/3 longer than the sepals, pale sulphur yellow to greenish, sessile, rounded, broadly spatulate, usually one of them apiculate; anthers 9–10 (rarely up to 13) mm. long, almost sessile; ovary 6-angled.—N.C., S.C., Ala, and Ga., locally found throughout, notably in pine woods.

2. **T. sessile** L. Species Plantarum, p. 340. 1753.
Solanum triphyllon Catesby. Nat. Hist. Carol. **1:** 50, pl. 50. 1754.
T. longiflorum Raf. Med. Fl. **2:** 96. 1830.
Phyllantherum sessile Nieuwland. Am. Mid. Nat. **3:** 113. 1913.

Stems smooth, relatively slender, 1–2 dm. tall; leaves oval to suborbicular, 4–8 cm. (or rarely up to 10 cm.) long, sessile, of one color or mottled, obtuse or acute, rounded at the base, often overlapping; sepals lanceolate, 2–3.5 cm. long; petals narrowly elliptic or sometimes oblong, sessile, acutish or obtuse, purple, maroon or brown; filaments dilated near the base; anthers 10–12 mm. long; ovary six-angled; berry broadly ovoid, 1–1.5 cm. long, red.—Pa. west to Minn., Ia., Mo. and Ark., south to Fla., Ala. and Miss. Most of the old reports of this species from the Carolinas are referable to *T. Underwoodii* or to the very common *T. Hugeri* which has but recently been distinguished. True *T. sessile* in the Carolinas is confined to the coastal plain and piedmont, its place in the mountains being taken by *T. Hugeri*.

Teratological specimens are very common. Polypetalous flowers, foliaceous stamens, single leaves from the rootstocks, very leafy stems, and variations in the size and number of all parts of the plant are not unknown.

T. sessile forma **luteum** (Muhl.) Peattie *n. comb.*
T. sessile var. *luteum* Muhl. Cat. 38. 1813.

Flowers yellowish or greenish.

This color form, for it is nothing more, was described by Muhlenberg without very satisfactory identity but it seems certain that he knew only *T. sessile* among the sessile-flowered species. I have seen no authentic specimens of this form from the Carolinas, but it is to be expected that it is found there.

3. **T. Underwoodii** Small. Bull. Torr. Bot. Cl. **24:** 172. 1897.

Rootstock creeping; stems smooth, stout, 1–2 (rarely 3) dm. tall; leaves not overlapping, lanceolate or ovate-lanceolate, rather abruptly acute or acuminate-acute, 10–15 cm. long, handsomely mottled with 3 shades of green; sepals lanceolate, 4–5.5 cm. long; petals elliptic or oblanceolate, 5.5–8.5 cm. long, sessile, acute or obtuse, erect, purple, maroon or brown; filaments less than 2 mm. long; anthers 1–1.5 cm. long; ovary 6-angled; berry ovoid, 1.5–2 cm. long, red.—N.C. south to Fla. and Ala., west to Tenn. In the Carolinas it is a coastal plain and piedmont, not a mountain plant.

4. **T. Hugeri** Small. Fl. S. E. U. S. A., p. 277. 1903.

Rootstock creeping; stem smooth, stout, 1–3 dm. tall; leaves broadly ovate to suborbicular, mostly overlapping, 10 (rarely 8) to 15 cm. long, as broad as long, of one color or often blotched with 2 shades of green; flowers sessile; sepals 4–5.5 cm. long; petals varying from 4–8 cm. long and 1–4.4 cm. broad, oblong-lanceolate to oblong-ovate, purple, maroon, or brown; anthers 10–20 mm. long; filaments over 2 mm. long; ovary 6-angled; berry broadly ovoid, 1.5–2 cm. long, dark red or purple.

In typical form the leaves are large and almost rhombic and abruptly acute and strongly overlapping; occasionally they vary towards the smaller, slender, acuminate, discrete leaves of *T. Underwoodii*, but the longer filaments appear to serve as constant character for differentiation. An occasional low specimen is difficult except on reproductive characters to separate from *T. sessile*, but the creeping rootstock is characteristic.—N. C. and Tenn. to Ala. and Fla. Especially common in the mountains of the Carolinas where it is by far the commonest of the sessile species. Nearly all old reports of *T. sessile* and perhaps some of those of *T. Underwoodii* from the mountains belong here, as does Rendle's description (Journ. Bot. **39:** 225. 1901) of a supposed variant race of "*T. sessile*."

The flowers of this species, like that of the two preceding are usually aromatic, the odor being apparently exuded by the petals and resembling that of *Calycanthus fertilis* and *Asimina triloba*. As the flowers fade, however, it may become rather rank.

T. Hugeri forma **flava** Peattie *f. n.*

T. luteum Harb. Bilt. Bot. Stud. **1:** 21. 1901. Not *T. sessile* var. *luteum* Muhl. on which Harbison based his transferred name.

T. Underwoodii var. *luteum* MacBride. Contrib. Gray Herb. n.s. **56:** 19. 1913. Also based on Muhlenberg's name which should be applied to *T. sessile.*

T. luteum var. *latipetalum* Gates. Ann. Mo. Bot. Gard. **4:** 46. 1917.

This form resembles the species except in having petals, stamens, and ovary sulphur or lemon yellow. It is locally found throughout the range of the species, and in southern Kentucky is much more abundant than the type according to Dr. Edgar T. Wherry.

Why a color form should have been given specific rank it is hard to see, since it lacks any other distinguishing characters. Gates notes that the petals may be greenish, or the petals may be purple and the anthers yellow; he suggests that these may be hybrids and he may be right, but they are probably not to be regarded so much as hybrids between separate species as crosses between color races of one species.

T. luteum var. *latipetalum* Gates is a variety based on House's specimen No. 1789 from Oconee, S. C., where the yellow petals are oblong-obovate and the anthers purple. The form of the petals is striking, but even in the normal maroon-colored petals there is variation from a spatulate-obovate type to an extremely long and slender type. Forms and varieties of this sort might be described without end in a variable genus like *Trillium* with small profit to systematic botany.

5. **T. viride** Beck. Am. Journ. Sci. **11:** 178. 1826.

Stems rough-pubescent near the top or glabrous at maturity, 1–2 dm. tall; leaves sessile, ovate to oblong or rarely suborbicular not overlapping, deep green, often blotched, acute, more or less pubescent on the veins beneath, sometimes abruptly short-attenuate at the base; sepals 2–4 cm. long; petals 2–4.5 cm. long, linear or linear-elliptic, clawed, light green or purplish green; filament 2–3 mm. long, flattened; anthers 1.5–2 cm. long; ovary 6-angled; berry ovoid, about 1.5 cm. long. —Mo. and Kans. to N. C., Ky., Va., Ala., and Miss. Chiefly a western plant, found in N. C. chiefly in the piedmont and mountains; I have seen no South Carolina specimens.

6. **T. pusillum** Michx. Fl. Bor. Am. **1:** 215. 1803.
T. pumilum Pursh. Fl. Am. Sept. **1:** 245. 1814.
T. texanum Buckl. Acad. Phil. Proc. **12:** 443. 1861.

Stem 1–2 dm. tall, sometimes mottled above; leaves 3–4 cm. long; blades oblong to lanceolate, sessile but cuneate at base; sepals lanceolate to oblong-lanceolate, about 1.5 cm. long; obtuse; petals white or pink, linear-oblong to lanceolate; anthers 5–6 mm. long, on filaments 2–3 mm. long.—In woods of the coastal plain and sometimes even to the mountains (2700 ft., Haywood Co., N. C.) in North and South Carolina; on the coastal plain of South Carolina this somewhat rare species has been noted from St. John's, Berkeley; Bluffton, Orangeburg.

7. **T. erectum** L. Species Plantarum, p. 340. 1753.
T. rhomboideum Michx. Fl. Bor. Am. **1:** 215. 1803.
T. foetidum Salisb. Parad. Lond., pl. 35. 1805.
T. erectum var. *atropurpureum* Pursh. Fl. Am. Sept. **1:** 245. 1814.
T. purpureum Kin in Ell. Sketch **1:** 430. 1817.
T. nutans Raf. Med. Bot. **2:** 99. 1830.
T. spatulatum Raf., l. c., 101.
T. atropurpureum Curt. ex. Beck. Bot. N. & M. States, p. 361. 1833.

Stems 3–6 dm. tall; leaves oval rhombic or more often broadly rhombic, 6–16 cm. broad, short acuminate, cuneate at base and sessile; peduncle 3–10 cm. long, erect or oftener horizontal or even declined; flowers ill-scented; sepals 2–3.5 cm. long, spreading, lanceolate or oblong; petals dark red (not purple) ovate-oblong or oblong-lanceolate, spreading, 3–4 cm. long, acuminate or rather blunt; filaments stout, 3–4 mm. long; anthers 5–9 mm. long, yellow; ovary 6-angled, dark purple: berry 6-angled, purple or nearly black, plump, about 2 cm. long.—Northern Ontario and Quebec to Man. and Minn. south to N. S. N. B. Mo., Ga., Ala., and Tenn. Perhaps the most widely distributed species of the genus; found frequently throughout North and South Carolina.

Polymerous flowers and foliaceous stamens are common aberrations in this species. The understanding of this species has varied much with different recent students. Species have been separated from it which had no constant differences, while varietal differences have existed which have been ignored.

Rendle (Journ. Bot. **39:** 228. 1901) has described from herbarium material in England two races with petals "white or claret" in both races. They are essentially as follows:

Race 1	*Race 2*
Smaller, less robust	Larger, more robust
Flowers small	Large flowers
Stamens barely or not much longer than the short recurved stigmas	Stamens shorter than the tips of the long outwardly curving stigmas
Petals slight to ½ longer than the sepals	Petals equalling the sepals
Stamens 6–10 mm. long	Stamens 10–20 mm. long

These "races" should be watched for in the field and if they can be supported they will upset all our present notions of the varietal divisions of this complex species. However, I have not found them to be of any value, so freely are the characters enumerated by Rendle interchanged and permutated.

T. erectum var. **album** (Michx.) Pursh.

T. rhomboideum var. *album* Michx. Fl. Bor. Am. **1:** 215. 1803.
T. erectum var. *album* Pursh. Fl. Am. Sept. **1:** 245. 1814.
Curt. Bot. Mag., pl. 1027. 1807.
T. nutans Raf. var. *bicolor* Raf. Med. Bot. **2:** 99. 1830.
T. Rugelii Rendle. Journ. Bot. **39:** 331, pl. 426, fig. b. 1901.
T. album Small. Fl. S. E. U. S. A. ed. 1, p. 278. 1903.
T. erectum f. *albiflorum* R. Hoffm. Proc. Bost. Soc. Nat. Hist. **36:** 244. 1922.

Petals white creamy yellow, or pink with purple lines; ovary white or pink or purple-black, filaments and connectives white, pink, or purple, flower often scentless; otherwise identical with the true species and like it varying in size and shape of petals and leaves.—Range of the species.

A highly variable group of plants. We are probably confronted with a miscellany of sports arising continuously and independently and perhaps hybridizing. Many variations may be found in the same plot of ground, and all attempts to make varietal difference are broken down by the inconstancy of linkage between the characters.

Gates (Ann. Mo. Bot. Gard. **4:** 53) discusses two series of variations: (1) A simple white-petalled form, differing in no other way from the species—a case of simple albinism, and (2) an extreme which differs not only in having white or pinkish petals which are less inclined to be

acuminate, but in its smaller flowers and larger anthers (8–11 mm. long), with pale connectives colored like the filaments. The second race he bases on Small's *T. album*. However, I am unable to find that any white flowered *T. erectum* plants have any smaller flowers than are often seen among red individuals, or that longer anthers always accompany small white flowers, or, indeed, that any of the characters remain linked.

An assemblage of color variants does not deserve specific rank and indeed one might reduce them all to a form if they were a little simpler, but in view of their relative complexity it may perhaps be as well not to make a new combination of names but to let them stand as var. *album*.

I. H. Hall (Bull. Torr. Bot. Cl. **1:** 21) considered var. *album* only an "unhealthy" state of *T. erectum*, and he bases this belief upon a supposition that white-flowered and red-flowered forms arise from the same root in different years, but he admits he does not know this to be true. I cannot gainsay this as regards western New York where Hall made observations, but it is not true in the Carolinas. If anything I find var. *album* to be more vigorous in all its organs than the true species and it is rarely that red and white flowers of this species are found anywhere in the same district.

In regard to *T. Rugelii* Rendle, which has caused much speculation among botanists, I may admit I have never seen the type. Rendle's figure however is not different from *T. erectum*, and his own description admits that it differs only in its "nodding flower and subrotund petals"—characters known to be irregular and inconstant in this species. The type specimen is said to have been collected in March, 1841, by Rugel from "the mountains at the Broad River, N. C." This is rather an indefinite locality but it is at least in western N. C., being either the French Broad which is west of the Blue Ridge, or the Broad, which arises in the Blue Ridge and flows southeast.

Among the many curious variants, too elusive for taxonomic standing, is one especially frequent in the mountains of North Carolina, which has purple blotches or veins on white petals. Gates (Ann. Mo. Bot. Gard **4:** 54) notes the same phenomenon from Oswego, Ithaca and Utica, N. Y., Cincinnati, O., Hennepin Co., Minn., Mt. Carmel, Ill., Port Huron, Mich., and I. H. Hall, loc. cit., notes this variation from New York. This is probably only a hybrid between color races.

T. erectum forma **viridiflorum** (Curt.) Peattie *comb. nov.*

T. erectum var. *viridiflorum* Curt. Bot. Mag., pl. 3250. 1833.

T. erectum var. γ *petalis ochroleuchis* Hook. Fl. Bor. Am. **2:** 180. 1840.

T. erectum var. *ochroleucum* Hook. ex. Macoun. Cat. Canad. Pl. **4:** 49. 1888.

T. pendulum Willd. Ges. Naturforsch. Fr. Berlin Neue Schr. **3:** 421. 1801.

Based upon a figure of Willdenow's with leaves acuminate instead of obtuse and smaller flowers, this rare form with greenish flowers is known with certainty only from Canada, but the likelihood of discovering it in mountainous Carolina in the course of more intensive field work is so great that it ought certainly to be mentioned here.

This concludes what is possibly only a partial sketch of the highly variable species *T. erectum*. Botanists should aid in collecting a full line of specimens accompanied by good notes and by publication of their information make complete our knowledge of this species.

8. **T. Vaseyi** Harbison. Bilt. Bot. Studies **1:** 24. 1901.

Stems smooth, 3–6 dm. tall; leaves oval or broadly oval, rather long-acuminate; pedicel horizontal or declined; sepals more or less curled above the middle; petals ovate to orbicular-ovate, 4–6 cm. long, of a rich red-purple color; flowers rose-scented; filaments slender, 7–12 mm. long, about equalling the anthers which have purple connectives; ovary dark purple, six-angled, the recurved stigmas much surpassed by the stamens.—Haywood and Transylvania Counties, high mountains of N. C. and Oconee Co., S. C., also reported from Tenn. and Ga. Gates says "Connecticut" but this seems improbable and his map does not show the range thus.

T. Vaseyi forma **album** House. Muhlenbergia **6:** 73. 1910.

Petals pure white, but anthers and pistils of the normal purple color. There is a specimen from Pigeon Gap, Haywood Co., N. C.

9. **T. simile** Gleason. Bull. Torr. Bot. Cl. **33:** 391. 1906.

Similar to *T. Vaseyi* but the leaves larger (1–1.8 dm. broad) broadly obrhombic, very short-acuminate; peduncle 3–7 cm. long; petals creamy white, broadly ovate or ovate-rhombic, obtuse or subacute, 3–6 cm. long, 2–4 cm. broad; filaments only 5–6 mm. long, about 1/2 as long as the wholly yellow anthers; ovary 6-lobed, dark purple.

Gleason says the peduncle is "declined or cernuous" but from my observations in the field it is erect; the flower itself may be declined or cernuous on the peduncle, generally at right angles to the peduncle. Deliciously fragrant, this species is quite the handsomest of the genus and is further very rare.—Polk and Buncombe Counties, N. C. (probably also Henderson Co.) Fannin Co., Ga. In N. C. found in deep cool mountain woods of the Blue Ridge at moderate elevations.

10. **T. cernuum** L. Species Plantarum, p. 339. 1753.

Stem slender, 2–5 dm. tall.

Leaves 3-nerved, broadly rhombic-ovate, acuminate, more or less cuneate at base; peduncle 8–33 mm. long, nodding below the leaves; petals 5–9 mm. broad, oblong-lanceolate, wavy, recurved-spreading; mature anthers 2.5–4.5 mm. long, scarcely longer than the filaments; stigmas stout, upwardly tapering; fruit ovoid, reddish purple.—In rich woods, Nova Scotia to Minn., Ga. and Mo. Rather rare in North and South Carolina as a mountain and piedmont plant.

11. **T. catesbaei** Ell. Sketch **1:** 429. 1821.

Solanum triphyllon; flore hexapetalo, carneo, Catesby. Nat. Hist. Car. **1:** 45, pl. 45. 1771.

T. cernuum L. Sp. Pl., p. 339. 1753. In part.

T. nervosum Ell. Sketch **1:** 429. 1821.

T. stylosum Nutt. Gen. **1:** 239. 1818.

Stem 2–5 dm. tall, mottled above; leaves 5-nerved, elliptical, rhomboid, or oval, 5–15 cm. long; pedicel spreading or deflexed, 2–5 cm. long; sepals linear or linear-lanceolate or sometimes spatulate-oblong, 2–4 cm. long, curled or reflexed; petals pink or sometimes rose, rarely whitish, oblong to oblong lanceolate, obtuse or abruptly pointed, 0.5–1.5 cm. broad and about 3 cm. long; stamens whitish; anthers curved or curled, 5–8 mm. long, on filaments of about the same length; berry 1–1.5 cm. thick, whitish. Flowers faintly fragrant.—Woods, especially the mountains, and to some extent on the piedmont, Ga. to Ala. and N. C., apparently rare in South Carolina.

12. **T. grandiflorum** (Michx.) Salisb.

T. rhomboideum grandiflorum Michx. Fl. Bor. Am. **1:** 216. 1803.

T. grandiflorum Salisb. Parad. Lond. Pl. 1805.

T. erythrocarpum Curt. Bot. Mag., pl. 855, 1805, not Michx.

Stems smooth and stout, 2–6 dm. tall; leaves broadly oval or rhombic, acuminate, 7–14 cm. long, more or less cuneate at the sessile or constricted base; peduncle erect, 5–8 cm. long; petals 2.5–5 cm. long, acute, erect-spreading (reaching 7 cm. in length and 3.5 cm. in width at times), much exceeding the narrower sepals, crisped, white; stamens with stout filaments (persistently green about the subglobose red fruit which is 2–2.5 cm. thick; anthers exceeding the very slender or suberect stigmas; ovary small, white.—Northern Quebec and northern Ont. to Minn., and south to Fla., Ala. and Ark., and west to Mo. and Ia. In N. C. and S. C. found chiefly in cool mountain woods.

An extremely beautiful species, sometimes confused with *T. erectum* var. *album*. It is the most variable species of the genus. Polymerism is very common, as are partial or complete viridism of petals, sterility of stamens, albinism partial or complete of the sepals. The variety *variegatum* E. T. Smith (Bot. Gaz. **4:** 180. 1897) is a monstrosity with green striped petals and petioled leaves, often seen in the Carolinas. Farwell (Ann. Rep. Mich. Acad. Sci. **20:** 155–160) has described from southern Michigan a number of monstrous forms, including one with no leaves at all. Farwell's forma *orbiculare* with green mucronate petals and a white border above the middle, and his forma *spatulatum* with spatulate-oblong to spatulate-cuneate petals with a green band down the middle and his *T. Chandleri* with no leaves, and five adjunct named forms based on color, number and size of petals, may all be regarded as a series of teratologies quite interesting as such but unworthy of standing in systematics. Many of these aberrations are known in the Carolinas as is Rafinesque's *T. lirioides* (Med. Fl. **2:** 100. 1830 which has petioled leaves.

T. grandiflorum forma **viride** Farwell. Ann. Rep. Mich. Acad. Sci. **20:** 157. 1918.

Petals all green. A simple variant and not a hybrid or monstrosity; deserving notice as it may well be found in our area; described from Michigan.

T. grandiflorum f. **roseum** Farwell. Ann. Rep. Mich. Acad. Sci. **21:** 364. 1919.

Flowers rose-colored. This raises the question of the fading of white *T. grandiflorum* petals to pink, as mentioned by Pursh under his *T. obovatum*. But Farwell states that the flowers which he characterizes

under this variety are always rose and the white ones are always white. In my observation there are indeed flowers that are roseate from the beginning, and others that remain white, but I have observed white petals commonly to fade pink.

T. grandiflorum var. **parvum** Gates. Ann. Mo. Bot. Gard. **4:** 58. 1917.

Has leaves generally more narrowly ovate, the floral parts all distinctly smaller and rather narrower, the petals spirally arranged, that is, overlapping at the base and curving outwards, forming a throat; flowers white or pale pink, fading deep pink or rose.—Probably found with the type throughout the range. It is common around Tryon, N. C.

Farwell has considered this identical with *T. obovatum* Pursh and has described it as *T. grandiflorum* var. *obovatum* (Pursh) Farwell (Rep. Comm. Parks & Boul. Detroit **11:** 53. 1900). It is difficult to know what Pursh meant by his description, but it does not seem to differ especially from normal *T. grandiflorum*, while Gates' characterization brings out many salient points unmistakable to those who know this plant in the field—the spiral arrangement of the small, narrow petals.

13. **T. undulatum** Willd. Ges. Naturforsch. Freunde Berlin Neue Schr. **3:** 422. 1801.
T. erythrocarpum Michx. Fl. Bor. Am. **1:** 216. 1803.
T. pictum Pursh. Fl. Am. Sept. **1:** 244. 1814.

Stem 2–5 cm. tall; leaves 6–20 cm. long, ovate, the petioles 3–20 mm. long; peduncle erect, 2–6 cm. long; sepals lanceolate to oblong-lanceolate, 1.5–2.5 cm. long; petals 1.5–2.5 cm. long, oblong to oval varying to obovate, white striped or ringed with pink or purple; anthers red, shorter than the stigmas, as long as the filaments, 4–5 mm. in length; berry bright red.—Generally in acid soil, Mo. to N. S. and Wis., and east to Ga. In the Carolinas it is a rare mountain plant; I know of it definitely only from Waynesville, Highlands, Roan Mt. and Blowing Rock, N. C., and Caesar's Head, Greenville Co., S. C.

LEE HEIGHTS, ROSSLYN, VA.

OTHER WATER MOLDS FROM THE SOIL

By W. C. Coker

Plates 27–36

This paper is a report of the work done by my class in mycology during the last five or six months. Members of the class have carried on the cultures, isolated the species, and drawn the figures, which in most cases were inked in by Miss Alma Holland. The students contributing are as follows: Mr. W. T. Alexander (*Dictyuchus achlyoides* and *Brevilegnia unisperma* var *delica*); Mr. H. H. Braxton (*Achlya inflata*, the two forms of *A. abortiva*, *Brevilegnia unisperma* var. *montana* and *Brevilegnia linearis*); Mr. A. B. Couch (*Achlya bisexualis*, a new heterothallic species); Miss Velma Matthews (*Leptolegnia eccentrica* and *Aplanes Treleaseanus*, the latter also isolated by two others).

Achlya bisexualis n.sp.

Plate 27

Plant heterothallic; growth vigorous on ordinary media, reaching a centimeter or more in a week on hemp seed or corn grain. Hyphae rather stout, about as in *A. flagellata*, primary sporangia long, pointed, commonly about 30–60 × 300–950μ; spores 9.6–10.8μ, behavior as usual for the genus. Gemmae typically pyriform to flask-shaped, often nearly spherical, some elongated, most 40–100μ thick; after a rest sprouting a tube (often long) and liberating spores directly or forming sporangia at the ends of the tubes. Oogonia borne on rather long (rarely short) laterals or apically on main threads, prevailingly spherical, not rarely oblong, about 50–80μ thick; the wall thin and apparently pitted only where the antheridia touch. Eggs 2–10, about 24–30μ thick, eccentric with one large oil drop, only rarely maturing. Antheridial branches long, much branched, abundant, producing a mass of filamentous, branched antheridia, which as a rule almost or quite cover the oogonial surface; no fertilization tubes yet seen.

Type male (No. 29) taken from a water collection from a small pond just below Lake View, N. C., Oct. 10, 1926. Type female (No. 17) from a water collection from Bowlin's Creek, just below the Durham road bridge, Sept. 21, 1926.

This is the second case reported of heterothallism in species belonging to the Saprolegniaceae, the other being the *Dictyuchus* reported by Professor J. N. Couch. Our new plant obviously falls in the *prolifera* group and seems to be nearest *A. flagellata*. Aside from its striking heterothallism it differs from that species in its less elongated gemmae and in the far more elaborate wrapping of the oogonia by the antheridia. It also lacks the irregular oogonia that so frequently appear in *A. flagellata*.

To all appearances this is the same plant as described in our *Saprolegniaceae*, p. 137, as *Achlya* sp., form without oogonia.

Last November Mr. A. B. Couch began an extensive series of crosses between a number of strains of sterile Achlyas that he and others had isolated during the preceding months. One of the first ten pairings of twenty sterile strains gave a conspicuous line of oogonia and antheridia on the line of contact, and we take this pair as representing the male and female sexes of a new species. Mr. Couch continued his work to include all possible crosses with each collection and also crosses with other Achlyas not hetherothallic. Unfortunately just as he was expecting to get his results ready for publication in this paper he became ill. I therefore include below only that small part of Mr. Couch's notes that were nearly ready for publication.

On Nov. 6, twenty sterile Achlyas were crossed to make ten pairings. In each case a sterile *Achlya* from around Chapel Hill was crossed with another sterile *Achlya* from Lake View or from territory in the vicinity of Lake View. They were grown on boiled cotton seed in water.

Forty-eight hours later the cultures were examined and abundant oogonia and antheridia appeared in the cross of the Chapel Hill *Achlya* No. 17 (female) and the Lake View *Achlya* No. 29 (male). The space in which oogonia appeared was strictly confined to the line between the two cotton seeds on which the two Achlyas were mingling.

The oogonia formed as a result of the crossing of these two sterile Achlyas were almost completely covered with antheridia which before applying themselves to the oogonia had in many cases twined about hyphae of the female plant and the oogonial stalk forming an intricate network.

On Tuesday, November 9, both male and female plants were discharging spores in abundance. The line of oogonia between the two seeds was still very distinct even to the unaided eye.

On Nov. 10, the line of oogonia was becoming indistinct and the

oogonia instead of developing, showed signs of going to pieces. A haze surrounded the antheridia applied to the oogonia which appeared as though the antheridia were in a state of decomposition.

On Nov. 11, the line of oogonia was indistinct and many oogonia were going to pieces. The region containing oogonia was still confined to a more or less narrow area between the two cotton seeds. The female plant had now taken on a decided new growth, but the male plant was not similarly stimulated. That part of the male plant most remote from the oogonial region had undergone no decided change in appearance.

On November 17, twelve separate isolations of sterile Achlyas from both soil and water were crossed with male strain No. 29 and female strain No. 17 in petri dishes on corn meal agar. None of these crossings with the female strain No. 17 produced any results. The male strain No. 29 induced oogonial production with Numbers 0, 17, 77 and 79. With collection No. 20 the antheridia-like branches from No. 29 coiled about the hyphae but no oogonia appeared. There was no response with collections Nos. 19, 21, 33, 42, 43 and 78.

On December 8, male strain No. 29 was crossed on corn meal agar in petri dishes with 16 still different isolations of sterile Achlyas from soil and water. From these crosses oogonia were formed in five cases (Nos. 5, 9, 10, 16 and 45). From the other 11 no response of any consequence was noted. Eight of the collections that showed no response with No. 29 were crossed with each other, but from these no results were obtained. Subsequently six of the numbers here crossed with No. 29 with no results were proved to be male by crossing with female strains.

Achlya abortiva forma **normalis**

Plate 28, figs. 1–7

Since our publication of *A. abortiva* in the last number of this Journal a very curious situation has arisen. The original pure one spore culture has been continued by transfers and remains unchanged. It has the mycelial growth of a normal looking *Achlya*. From the same collection of soil that gave this species we soon afterward isolated two other plants. One of these is a very distinct species published below as *A. inflata*. It is the other plant that gave such peculiar results. It was secured on a hemp seed and to the naked eye had all the appearance of *Geolegnia*, the growth being limited and very dense. Under magnification it was shown to be a contorted, gnarled, abnormal-looking

Achlya with very irregular oogonia and no antheridia. A typical looking bit of this growth is shown on plate 28, fig. 8. Several single hypha cultures were made from this original and in every case the result was a normal looking *Achlya* with average growth, nearly regular oogonia and plenty of diclinous antheridia. For example, this result was obtained from the piece shown in fig. 8, which was itself put on agar after being drawn and gave the same plant as the spores, the hyphae springing from the central gemma-like joint. All transferences continue to give the same normal plant, but the same curious original continues to live. On carefully comparing this last with *A. abortiva* we are convinced that it is a strange form of that species. It then follows that *abortiva* itself is a form of the more normal plant with antheridia and good eggs that we now have. We would like to give this last a specific name and call *abortiva* a form of it, but as this seems to be against the rules, we must call the new plant a normal form of *abortiva*. In answer to a natural question it may be said that we have kept a sharp lookout for parasites in all these forms as well as in *inflata* and have seen no evidence at all of any attacking organisms either inside or outside the plants. Below is a description of the normal form above mentioned:

Growth not very dense, reaching a length of 8 mm. on hemp seed or bits of boiled corn. Main hyphae slender, up to 115μ at the base, averaging 12–24μ throughout the central portion of growth. Sporangia terminating the main hyphae, not abundant, soon disappearing, sparingly reproduced by cymose branching, up to 720μ long, usually 370–550μ, slender, occasionally containing as few as two rows of spores, broadest near the distal end. Spores 11.5–12.5μ in diameter, behaving as usual for *Achlya*. Gemmae fairly abundant in old cultures, varying in shape and size, rod-shaped or clavate to oval, up to six or more in a row. Oogonia abundant in most cultures, especially on corn, terminating the main hyphae or borne on short lateral branches usually $\frac{1}{2}$–2 times as long as the diameter of the oogonia, two or more often borne on a lateral stalk, usually spherical, often somewhat irregular, 30–98μ in diameter, mostly 55–75μ. Oogonial walls about 3μ thick, with numerous conspicuous pits, often giving the wall a wavy appearance. Eggs 1–5 to an oogonium, usually 2–4, 21–45μ in diameter, averaging 31–36μ, with walls 3–3.3μ thick, eccentric, with a large oil drop at one side of the egg, the great majority of eggs reaching normal maturity. Antheridial branches long and slender, always declinous, soon disappearing; antheridia one or more on most of the oogonia, becoming less noticeable as the culture ages because of the early disintegration of the antheridial branches.

Achlya abortiva, contorted form.

Plate 28, figs. 8 and 9

The peculiar abnormal form from which the above described plant was obtained may be described as follows:

Growth on hemp seed very short, dense, and slow, much like *Geolegnia*, reaching a length of 4 mm. Hyphae short and irregular, gnarled, much branched. No sporangia noticed. Gemmae rough or irregular, spherical or clavate. Oogonia rather numerous, borne on the tips of main hyphae or on short lateral branches, very irregular in shape, usually somewhat spherical, with numerous outgrowths; from 40–120μ in diameter, walls fairly thick, unpitted. Eggs 1–5, usually 1–3 to the oogonium, spherical, often made somewhat irregular by oogonial pressure, 32–45μ in diameter, eccentric, many of them going to pieces before maturity. No normal antheridia seen.

When transfers by hyphae and gemmae were made from this culture to agar, hemp seed, corn, etc., the normal form of *Achlya abortiva* was obtained in all cases.

Collected once from dry black soil $\frac{1}{2}$ in. deep under moss in mixed woods near Southport, N. C., May 15, 1926, growing with *Achlya abortiva* (type) and *A. inflata*.

Achlya inflata n. sp.

Plate 29

Vegetative growth fairly dense on boiled hemp seed or morning glory seed, reaching a length of 14 mm., growth very poor on corn. Main hyphae not large, up to 75μ at base, averaging 15–25μ throughout the culture; growing tips smooth, hyaline. Sporangia not very abundant, found rarely in old cultures, secondary ones not usually formed; about the same width as the hyphae bearing them and up to 375μ long. Spores 10.5–11.5μ in diameter, forming an irregular group at the mouth of the sporangia and behaving as a typical *Achlya*. Gemmae found in all older cultures, though not very abundant, generally long-clavate to spherical, often up to five or six in a row. Oogonia abundant in all cultures, appearing after two or three days, though the contents of many of them disintegrate before forming eggs; large, globular, with an inflated appearance, 78–176μ in diameter, averaging 120–150μ, terminating long lateral stalks from the main hyphae, the stalks 2–3 times as long as the diameter of the large oogonia, sometimes shorter. Oogonial wall with several rather conspicuous pits, rarely with one or two blunt wart-like outgrowths near the base. Eggs rather numerous, from 2–20 to the oogonium, averaging 7–12, rarely filling even half the oogonium, 29–37μ in diameter, averaging 31–35μ, ec-

centric, with a large oil drop at one side, the majority disorganizing before maturity. Antheridial branches long, slender, always diclinous, not traceable even remotely to hyphae-bearing oogonia, touching each oogonium with one to several somewhat tuberous antheridia, soon disappearing. Antheridial tubes sometimes visible.

Found once, growing with *Achlya abortiva* in dry black soil ½ inch deep under moss in mixed woods near Southport, N. C., May 15, 1926.

The species is easily characterized by the very large oogonia, strongly diclinous antheridia and eccentric eggs which occupy only a small part of the oogonial space. The large oogonia (largest of any *Achlya*), small space occupied by the eggs (smallest in proportion of any species in the family), and the strong tendency to disorganization of the eggs, would indicate a close relationship to the *oblongata-apiculata* group. The egg structure, however, is different, and we have a suspicion that these resemblances are superficial, as in every respect except the inflated oogonia and numerous eggs, the species is exactly like the normal form of *A. abortiva*. Is it possibly a mutant of that plant which as seen above has the habit of acting strangely? If so, the mutation in form is more extreme than that recorded so far for any fungus. The plant has remained constant under numerous transfers for nearly a year at the present writing.

Brevilegnia Coker and Couch, New Genus

Mycelium depauperate, dense and opaque, never aerial; sporangia in the great majority behaving about as in *Thraustotheca*, the wall soon disappearing (in some species sporangia of the *Achlya* type also occur). Spores very variable in size and shape in the same culture, the larger ones multinucleate, encysting in position (except in the achlyoid sporangia) and only slowly separating after the disintegration of the sporangial wall; after encystment either emerging and swimming once or not swimming, depending on the species. Oogonia small, with a single eccentric egg. Antheridia present or wanting, prevailingly androgynous. Gemmae wanting in most species.

The dense, limited mycelium is a marked generic character and in some species is nearly as extreme as in *Geolegnia*. This, with the variable spore size, frequent suppression of motility and small oogonia with single eggs, indicates a kinship to that genus. On the other hand, *Brevilegnia* approaches nearest to *Dictyuchus* in sexual reproduction and to *Thraustotheca* in asexual reproduction. *Geolegnia* is separated by its consistently large, multinucleate spores and the long retention of the sporangial wall. All the species and varieties here included have been found only in the soil.

We are transferring to this genus the plants published in the last Mitchell Journal (**42**: 140–141. 1926) as *Thraustotheca unisperma* and its variety *litoralis*. The names therefore appear now as *Brevilegnia unisperma* Coker and Braxton and *Brevilegnia unisperma* var. *litoralis* Coker and Braxton. The type species is *B. subclavata*. (For key see p. 220.)

Brevilegnia unisperma var. **montana** n. var.

Plate 30, figs. 1–9

Last summer we isolated from the soil in the mountains of western North Carolina a plant that we are considering a variety of *B. unisperma*. It differs from the type in the lack of antheridia, and from both the type and the var. *litoralis* in prevailingly less irregular and warted oogonial wall and in the production of the oogonia in groups on long, branched laterals.

The plant is easily separated from all published species except *B. unisperma* by obvious characters, as very small oogonia with few eggs and absence of antheridia. The other species and varieties of *Brevilegnia* described in this issue all have small oogonia with single eggs, but they are all easily separated from this by such characters as presence of antheridia, or size, shape and behavior of sporangia or spores. The description follows:

Growth fairly dense on boiled hemp seed, jimson weed seed, or corn, reaching a length of 5 mm. Main hyphae freely branched, up to 75μ thick at the base, averaging from 15–30μ throughout the cultures. Sporangia and spores, as in the species, usually densely grouped in old cultures. Oogonia plentiful in most old cultures, appearing after about 10 days, borne as a rule on long, slender, loosely-branched hyphae about 6μ thick, which arise from the main hyphae. Oogonia obovate or oblong to subspherical, usually somewhat irregular or tuberculate and rarely prettily papillate, 17–26 $\times$ 18–32μ, averaging 20–23 $\times$ 25–30μ; oogonial wall thin, unpitted. Eggs spherical, sometimes elliptic, 12–24μ in diameter, averaging 17–19μ, usually single, rarely two to the oogonium, eccentric, with rather thick walls. No antheridia present.

Collected once from rich black loam 1 inch deep under pasture, grass, Springdale Farm, Haywood Co., N. C., July 24, 1926. (Altitude about 3000 feet).

Brevilegnia unisperma var. **delica** n. var.

Plate 31

Mycelium rather dense, reaching a length of 6 mm. on hemp and corn. Hyphae delicate, 15.2–21μ thick in main part of the culture. Sporangia extremely abundant, 94–682μ in length; many with only one row of spores, others with two rows (surface view), often with one row below and two above; primary sporangia borne on main hyphae, secondary ones by cymose branching and often from lateral branches of various lengths at some distance below. Spores more or less angular, 11.7–16.2μ, most about 12.5μ in diameter, both extremes occurring in the same sporangium; sporangial wall disappearing soon after maturity, but the spores while spreading a little remaining united for a long time as if in a jelly. Spores in the great mass without a swimming stage: a few, however, emerge about as in *Dictyuchus* and swim under normal laboratory conditions. Oogonia abundant throughout the culture, small, borne on long, slender, usually curved oogonial stalks which arise from main hyphae which in the great majority of cases bear only oogonia but may bear a sporangium at the tip. The oogonial stalks vary in length from about 3 to 7 (usually 3.5–4) times the diameter of the oogonia. Rarely an oogonial stalk may branch and bear two oogonia. Oogonia globular, with or without an apical projection, 16.5–21μ, mostly 18μ in diameter; wall smooth, unpitted. Eggs one in each oogonium, 15–17.5μ in diameter, eccentric with a single large oil drop. No antheridia present.

Found once in sandy loam soil at a depth of three inches near a branch in the area to be covered by the new stadium, Chapel Hill, N. C., Oct. 9, 1926 (Alexander, coll.).

The description is drawn from the plant as it appears in a single spore culture on hemp seed in distilled water. It also grows well on bits of boiled corn grains, jimson weed seed, etc.

Brevilegnia linearis n. sp.

Plate 32

Growth limited, dense and opaque as in other species of the genus, the hyphae reaching a length of 5 mm. on hemp seed or jimson weed seed. Hyphae slender, about 8–14μ thick throughout the culture, sparingly branched; growing tips even, hyaline. Primary sporangia terminating all the main hyphae, occasionally intercalary, long, slender, sometimes branched; secondary ones usually shorter, borne on the tips of lateral branches formed by cymose branching of the main hyphae. Sporangia of about the same thickness as the hyphae bearing them, with spores in a single row, up to 60 in a row, usually 10–24; the sporangial wall soon disappearing, but spores remaining held together for a long time by some invisible substance. Spores spherical to long rod-

shaped, all with a central vacuole that varies with the spore size, primary ones mostly subspherical, those of secondary sporangia more rod-shaped, 8–20 x 15–60μ, mostly 8–14 x 15–25μ, never swimming but sprouting in position. Oogonia fairly abundant, borne singly on long, slender, often coiled lateral branches from the main hyphae; typically spherical, sometimes slightly irregular, 16–21μ in diameter; wall thin, smooth, colorless. Eggs one to each oogonium, spherical 14.5–18μ in diameter, eccentric with a large lateral oil drop. Antheridia on nearly all oogonia, single, large in proportion, short and tuberous, usually androgynous from near the oogonium, rarely diclinous. Antheridial contents often entering the oogonium before the egg is differentiated.

In moist clay one inch deep under lichens in an open pasture near Saxapahaw, N. C. (Braxton, coll. Type).

We are placing this species in the genus *Brevilegnia* on account of the prevalence of small spores borne many to a sporangium and the early disappearance of the sporangial wall. Of the five species here placed in *Brevilegnia* the present one is apparently nearest *Geolegnia*, which it approaches in the universal occurrence of spores in a single row. In the prevalence of elongated spores it also approaches *G. septisporangia*.

Leptolegnia eccentrica n. sp.

Plate 33

Hyphae very small, 4.8–7.2μ thick, sparingly branched, forming a ring growth of 2 cm. on hemp seed in water in 10 days. Sporangia long, filamentous, same size as the hyphae, first ones usually unbranched, later ones may be branched, the spores escaping at the apex of any branch; spores in a single row, spherical, oval or elongated in the same sporangium, usually about 6μ thick and up to 16.8μ long, swimming away upon escaping then encysting with a diameter of 7.2–9.6μ, the majority about 7.2μ, and swimming again. Oogonia abundant, spherical to oval with numerous short irregular projections, including these projections 19.2–36μ in diameter, most from 24–28μ, borne on lateral branches, wall hyaline, very thin. Eggs one to an oogonium slightly irregular, spherical to oval, 14.4–27.6μ thick, most 22–26μ, eccentric with a large oil drop on one side; wall extremely thick, up to 5μ or more, consisting of a dark outer portion, lighter irregular central portion and a clear inner portion. Antheridia androgynous arising from the oogonial stalk, often from immediately below the oogonium.

Found three times, once from sandy soil under a beech tree at a depth of 1 in. near the Meeting of the Waters, Chapel Hill, N. C., Nov. 11, 1926 (type), once from damp sand at depth of 1½ in. from near the Meeting of the Waters, Nov. 11, 1926, and once from black sandy humus

at a depth of 2 in. about 100 yards from the lake at Lakeview, N. C.; Nov. 20, 1926.

This may be distinguished from the other two known species by the small oogonia and eggs with a single large oil drop and the closely androgynous antheridia. The very thick egg wall and papillate oogonial surface relate the plant to *L. subterranea* rather than to *L. caudata*. In our book "The Saprolegniaceae" the oogonial wall and egg wall of *L. caudata* are not very clearly shown. As a matter of fact the two walls are of almost equal thickness, the egg wall only about one-third as thick as in the other two species.

Sporangia begin forming in cultures on hemp seed when about a week old and continue for some time so that in old cultures almost the entire mycelium becomes cut up into spores. All the spores which were observed escaping came out at night, reshaping themselves as in the other species of the genus, and swam away. Some remain in the sporangium and sprout.

Oogonia begin to form in cultures from 7–10 days old, taking from 3–4 weeks to mature the eggs.

The egg is the most remarkable part of this plant as its structure seems to be different from any other of the Saprolegniaceae so far observed. It consists of a central portion with a large oil drop surrounded by granular protoplasm and an extremely heavy wall consisting of several parts which are very difficult to interpret. The outer part is thin, dark and irregular, the central portion is thicker and lighter and has a peculiar clotted look with different refractions, while the inner part is hyaline, usually thin but varying in the different eggs. The description and drawings are from a single spore culture.

Aplanes turfosus (Minden) n. comb.

In our book "The Saprolegniaceae," page 79, we referred to *Saprolegnia Treleaseana* Humphrey (as *Aplanes Treleaseanus*) a plant found by us in eastern North Carolina. We have recently found a plant differing from the one earlier described and now believe this last to be the true *S. Treleaseana* Humphrey. Humphrey's scanty description was taken from a preparation made by Trelease and the sporangial characters and spore behaviour were left in much doubt. [Kauffman says that the Michigan plant referred by him to this species has sporongia of the *Achlya* type.] The oogonia are however so like those of our new plant that we are convinced that the two are the same. We are now able to give a full account of the sporangia and feel that the plant with good

reason should be retained in *Aplanes* as first suggested. Our original plant is to all appearance *Saprolegnia turfosa* (Minden) Gaumann, which we are now transferring to the genus *Aplanes*. Synonyms of this are as follows: *Saprolegnia monoica* var. *turfosa* Minden, *Saprolegnia* sp. 2 Reinsch, and *Saprolegnia paradoxa* Petersen. For literature references of the above see "The Saprolegniaceae."

Our description of *Aplanes Treleaseanus* follows:

Aplanes Treleaseanus (Humphrey) Coker.
Achlya Treleaseana (Humphrey) Kauffman

Plates 34 and 35

Hyphae moderately stout, majority 28–48μ thick, sparingly branched. Growth slow but forming a heavy ring growth about 2 cm. in diameter on hemp seed in distilled water in three weeks. Sporangia rare, lacking in ordinary cultures, sometimes formed in old cultures on branches arising from gemmae or directly from gemmae, very variable in size, 12–30 $\times$ 100–500μ, spores in a single row or in several, spherical to cylindrical, 12–14.4μ thick and up to 16.8μ long, in the majority of cases sprouting *in situ*, also escaping as in *Achlya* or rarely as in *Dictyuchus*. Gemmae very abundant, rod shaped to oval in long chains, giving off numerous branches in old cultures, these branches usually cutting off one or more gemmae or rarely forming intercalary or apical sporangia. (Compare *Achlya Klebsiana*, Saprolegniaceae, plate 40, fig. 3.) Oogonia abundant when grown on hemp seed, varying greatly in shape from spherical to cylindrical, wall hyaline, pitted, 2–4μ thick, usually with numerous papillae 2–24μ or more long, occasionally smooth, spherical and oval ones 30–88μ in diameter, usually about 84μ, other oogonia 50–72 $\times$ 132–577μ, usually borne terminally on main hyphae or terminating a chain of gemmae, not rarely on short lateral branches; intercalary ones plentiful, also occasionally borne 2 or 3 in a chain. Eggs 1–25, usually 6–10 to an oogonium, 28.8–36μ in diameter, majority 31–36μ, spherical or often elliptic or block-shaped from pressure, filling the oogonium, subcentric with a sheath of small oil drops surrounding all but a small portion of the protoplasm; wall thin. Antheridia androgynous, arising from the oogonial stalk, often with the antheridial cell not cut off as in *A. turfosus*.

Studies were made with cultures from a single hypha taken from the collection first mentioned below:

Collected six times from Lakeview, N. C., on Nov. 20, 1926, by the class in mycology: (1) In wet black humus near the lake, (2) in wet muck under grass, (3) in black sand under *Lycopodium*, (4) in black peat under moss and (5 and 6) in water from the lake.

The only sporangia obtained were formed in two old cultures. The

sporangia which vary greatly in size were formed in most cases on the tips of branches from the gemmae and also directly from the gemmae. The gemmae which are formed in cultures a few days old, form numerous secondary gemmae on branches when the culture becomes older giving it a densely flocculent appearance to the naked eye. The branches which may be long or short absorb practically all of the protoplasm from the gemmae, and the secondary gemmae or sporangia formed at their tips in their turn absorb all of the protoplasm from the branches (compare the sprouting teleutospore of the rusts). The spores are arranged in a single row in the small sporangia but in the larger ones in several rows. The majority of the spores sprout *in situ* but in a great many cases the spores escape from the sporangium encysting in a clump at its mouth, then emerging from their cysts and swimming again as in *Achlya* or sprouting directly without a second swimming stage. The spores were not seen actually escaping from the sporangium but the clumps of spores conspicuous at the mouths of the sporangia for a little while after they had escaped, which was at night, were seen, and the next morning numerous empty cysts were present at the mouths of the sporangia and also many sprouting spores that had not escaped from their cysts. The spores which had emerged from their cysts were seen swimming very actively and were clearly of the form characteristic of the second swimming stage.

Oogonia formed in cultures on hemp seed in about 10 days forming mature eggs in 2–3 weeks.

The percentage of antheridia varied considerably in the different collections (about 1–50%), but all of those observed were androgynous.

Dictyuchus achlyoides n. sp.

Plate 36

Growth moderately dense on boiled hemp seed, reaching a length of 6 mm. in five days. Main hyphae from 21–45μ thick at base, gradually tapering toward the end. Sporangia arising from tips of hyphae and by cymose branching; both *Achlya* and *Dictyuchus* types abundant, the *Achlya* type appearing first, the *Dictyuchus* ones later. In old cultures the latter strongly predominate. Sporangia frequently branched, occasionally borne in rows. Spores 10–16μ, mostly 12.5μ in diameter, the same size in both types of sporangia, occasionally sprouting in place. Oogonia plentiful, spherical, 25–37.5μ in diameter, borne singly on long, slender, usually curved stalks which arise from the main hyphae and vary in length from about two to four times the diameter of the

oogonia. Oogonial walls unpitted, slightly yellow, provided with many papillae which vary in length from 3–11μ. Eggs eccentric, 20–27μ in diameter, mostly 25μ; one in each oogonium. Antheridia androgynous, usually arising from the oogonial stalk, occasionally from the main hyphae, appearing (singly) on about 65% of the oogonia.

Collected Jan. 1, 1927, on W. T. Alexander's farm near the spring at Mallard Creek bridge from sandy soil at a depth of three inches, Derita, N. C. (Mecklenburg County, type). Again on Jan. 1, 1927, from red clay soil at a depth of one inch in the barnyard of the same farm a half mile from the first station.

In reference to the two types of sporangia, it may be said that single-spore cultures were made from both types and in each case the spores gave rise to cultures which had both types of sporangia and the characteristic oogonia.

Calyptralegnia Coker, New Genus

In thinking over the classification of the water molds, much disturbed and complicated by recent discoveries, we believe that the situation can be considerably improved by the recognition of one other genus. By the removal from *Thraustotheca* of *T. achlyoides* and *T. unisperma*, that genus is left with clear and unequivocal characters, as internal encystment of the spores (at least in the greater part), extensive, diffused dehiscence or deliquescence of the sporangial wall, and multiple, eccentric eggs.

We would define the genus *Calyptralegnia* as follows:

Mycelium of the usual achlyoid type. Spores encysting within the sporangium, therefore angular; sporangium dehiscing by the breaking off of an apical segment, the spores escaping intermittently by the swelling of consecutive groups from above downward, afterwards emerging from their cysts and swimming as in *Achlya*. Eggs multiple, centric or subcentric with numerous small oil drops. Antheridia androgynous.

The type species, and at present the only species, is *Calyptralegnia achlyoides* Coker and Couch (see *Thraustotheca achlyoides*, this Journal **39:** 112. 1923).

The name indicates the dehiscence of the sporangium by the breaking off of an apical segment.

We are adding below a key to all the Saprolegniaceae in which the spores in great measure encyst within the sporangium.

Spores, at least in great majority, normally encysting within the sporangium; eggs eccentric. [It would probably be better to place *Achlya dubia* here as a *Thraustotheca*, but the achlyoid sporangia are often as numerous as the thraustothecoid ones.]

Eggs centric or subcentric with many peripheral oil drops
Calyptralegnia achlyoides

Eggs eccentric

Oogonia with more than one egg

Sporangia usually entirely wanting (*Aplanes*)

Oogonia strongly papillate.....*A. Treleaseanus*

Oogonia usually not papillate.....*A. turfosus*[1]

Sporangia abundant (*Thraustotheca*)

Oogonia smooth; all sporangia thraustothecoid.....*T. clavata*

Oogonia papillate; primary sporangia achlyoid.....*T. primoachlya*

Oogonia with a single egg.

Mycelium not depauperate but of usual water mold type. Spores not conspicuously of different sizes. Spores after encystment retaining their positions (sporangial shape retained). Spores normally with a swimming stage.....(*Dictyuchus*)

No sporangia achlyoid; oogonia smooth; antheridia diclinous or plants heterothallic.....*D. Magnusii*, (*D. sterile*, ect.)

No sporangia achlyoid; oogonia smooth; antheridia androgynous
Dictyuchus sp.
(This Journal **42**: 144. 1926).

Some sporangia achlyoid, oogonia papillate; antheridia androgynous
D. achlyoides.

Mycelium depauperate, dense and opaque.

Spores after encystment more or less spreading and separating and rounding up, the sporangial wall disappearing; spore size variable in same plant, many small subspherical ones; spore behaviour also varied, non motile or once motile (*Brevilegnia*).

No sporangia achlyoid; gemmae lacking.

Spores normally swimming once.

Antheridia present.....*B. unisperma*

Antheridia lacking.

Oogonia borne on loose proliferating branches
B. unisperma var. *montana*

Oogonia usually borne singly on short slender branches from the main hyphae.

Sporangia long, slender, a few *Dictyuchus*-like; some spores with and many without a swimming stage.
B. unisperma var. *delica*

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[1] The behavior of the spores in this species is still doubtful, as very few sporangia have ever been seen. What evidence there is, however, is to the effect that the spores leave the sporangia before swimming.

Sporangia shorter, entirely thraustothecoid; most of the spores with a swimming stage.....*B. unisperma* var. *litoralis*
Spores normally not swimming. (In some species may be made to swim by special treatment.)
Sporangia short, subclavate.....*B. subclavata*
Sporangia elongated, some but not the majority with spores in a single row.....*B. diclina*
Sporangia all with a single row of spores, many of which are elongated.....*B. linearis*
Primary sporangia achlyoid; gemmae present.....*B. bispora*
Spores large, subspherical, oval or elongated, multinucleate, sporangial wall retained and obvious. Spores always non-motile and in a single row.....(*Geolegnia*)
Sporangia constricted at intervals, spores spherical to oval *Geolegnia inflata.*
Sporangia not constricted, spores elongated *Geolegnia septisporangia.*

Below we add a list of the water molds of the families Saprolegniaceae and Blastocladiaceae that have so far been found in the soil. Aside from two parasitic species of *Aphanomyces*, none of these have been found in the soil except by us (Botanical staff and students of the University of North Carolina and Prof. Harvey). This includes those listed by Dr. Couch on p. 238.

Achlya americana, (3 times) Moore County, N. C.
Achlya abortiva, once, *A. abortiva* contorted form, once, and *A. abortiva* forma *normalis*, once from the same collection from Southport, N. C.
Achlya apiculata, once from Charlotte, N. C.
Achlya bisexualis, several females from Chapel Hill and several males from Chapel Hill and Moore County, N. C.
Achlya caroliniana, (41 times) Chapel Hill, Charlotte and High Point, N. C., and from Cold Spring Harbor, L. I., and Abbey Green, Jamaica.
Achlya colorata, (7 times) Chapel Hill, Wilmington and Moore County, N. C.
Achlya conspicua, once from Chapel Hill, N. C.
Achlya flagellata, (60 times) Chapel Hill, High Point, Beauford, Goldsboro and Wilmington, N. C., and Charlottesville, Va.
Achlya glomerata, (7 times) Chapel Hill, N. C., and Cold Spring Harbor, L. I., N. Y.
Achlya hypogyna, once from Chapel Hill, N. C.
Achlya imperfecta, (3 times) Chapel Hill, N. C.; Cold Spring Harbor, L. I., and Abbey Green, Jamaica.
Achlya inflata, once from Southport, N. C.
Achlya orion, (5 times) Chapel Hill and Lakeview, N. C.
Achlya racemosa, (3 times) Chapel Hill and High Point, N. C.
Achlya subterranea, (5 times) Warsaw, Southport and Wilmington, N. C.
Achlya sp., once from Jamaica, B. W. I. (Couch, unpublished).

Aphanomyces euteiches, twice from Chapel Hill and Haywood County, N. C.
Aphanomyces laevis, once from Chapel Hill, N. C.
Aphanomyces scaber, once from Cold Spring Harbor, L. I.
Aphanomyces stellatus, (4 times) Chapel Hill and Wilmington, N. C.
Aplanes Treleaseanus, (4 times) Lakeview and Wilmington, N. C.
Aplanes turfosus, (3 times) Wilmington, Newbern and Southport, N. C.
Brevilegnia bispora, once from Cold Spring Harbor L. I.
Brevilegnia diclina, (8 times) Madison, Wis.
Brevilegnia linearis, once from Alamance County, N. C.
Brevilegnia subclavata, once from Cold Spring Harbor, L. I.
Brevilegnia unisperma, (8 times) Chapel Hill and Aberdeen, N. C.
B. unisperma var. *delica*, once from Chapel Hill, N. C.
B. unisperma var. *montana*, once from Haywood County, N. C.
Calyptralegnia achlyoides, once from Chapel Hill, N. C.
Dictyuchus achlyoides, twice from Charlotte, N. C.
Dictyuchus sterile, (15 times) Chapel Hill, Haywood County and Moore County, N. C.
Dictyuchus, (homothallic form), once from Chapel Hill, N. C.
Geolegnia inflata, (10 times) Chapel Hill, Johnson County and Wilson County, N. C., and Cold Spring Harbor, L. I.
Geolegnia septisporangia, (5 times) Chapel Hill, Johnson County and Wilson County, N. C., and Cold Spring Harbor, L. I.
Isoachlya eccentrica, twice from Chapel Hill and Lakeview, N. C.
Leptolegnia eccentrica, twice from Chapel Hill and Lakeview, N. C.
Leptolegnia subterranea, (13 times) Chapel Hill, Lakeview, N. C., and Cold Spring Harbor, L. I.
Pythiopsis cymosa, (5 times) Chapel Hill and High Point, N. C.
Pythiopsis intermedia, (6 times) from Chapel Hill, N. C.
Saprolegnia asterophora, once from Chapel Hill, N. C.
Saprolegnia ferax, (3 times) Chapel Hill, Aberdeen and High Point, N. C.
Saprolegnia litoralis, (4 times) Moore County, N. C.
Saprolegnia (sterile form), once from Charlottesville, Va.
Thraustotheca clavata, (33 times) Chapel Hill and Haywood County, N. C., Cold Spring Harbor, L. I., and Abbey Green, Jamaica.
Thraustotheca primoachlya, (3 times) Beaufort and Pamlico County, N. C., and Shawnee, Okla.
Allomyces arbuscula, (9 times) Chapel Hill, Charlotte and Jones County, N. C., and Cold Spring Harbor, L. I.
Allomyces moniliformis, (4 times) Smith Island, near Smithfield, and by Pigeon River in Haywood County, N. C.

CHAPEL HILL, N. C.

EXPLANATION OF PLATES

PLATE 27

ACHLYA BISEXUALIS

Fig. 1. Young oogonium surrounded by an intricate mass of antheridia.
Fig. 2. Older oogonium with egg initials. Only antheridia on sides are shown; those over upper face are omitted.
Fig. 3. Oogonium with egg initials going to pieces.
Fig. 4. Oogonium with 2 ripe eggs and one going to pieces.
Fig. 5. Typical group of gemmae and an empty sporangium.
Fig. 6. Gemma forming spores directly.
Fig. 7. Gemmae forming long germ tubes with sporangia and secondary gemmae at their tips.
Fig. 8. Primary sporangia from a young culture.

Figs. 1–3 × 220; fig. 4 × 307; figs. 5–8 × 90

PLATE 28

ACHLYA ABORTIVA NORMALIS

Fig. 1. Habit sketch. Culture passed maturity and antheridial branches largely disorganized. × 143.
Fig. 2. Young oogonium with antheridia. × 143.
Fig. 3. Sporangia. × 55.
Fig. 4. Gemmae. × 143.
Figs. 5 and 6. Oogonia. Fig. 5 × 600; fig. 6 × 143.
Fig. 7. Oogonium with antheridium and immature eggs. × 347.

Achlya abortiva, contorted form

Fig. 8. Irregular gemma bearing 4 oogonia, one with contents disorganizing. × 347.
Fig. 9. Oogonium with 2 immature eggs. × 239.

PLATE 29

ACHLYA INFLATA

Fig. 1. Sporangium, oogonium and antheridia.
Fig. 2. Young oogonium with antheridia.
Figs. 3 and 4. Mature oogonia with antheridial branches disorganized.
Fig. 5. Abnormal behavior of young oogonium which is producing a number of abnormally small oogonial initials on which are antheridia.
Fig. 6. Gemmae.
Fig. 7. Oogonium with contents disorganizing.

Figs. 1–4 and 6 × 143; fig. 5 × 95; fig. 7 × 239

PLATE 30

BREVILEGNIA UNISPERMA var. MONTANA

Fig. 1. Habit of oogonia.
Fig. 2. Spore escaping from cyst.
Figs. 3 and 4. Sporangial habit.
Figs. 5–9. Oogonia showing immature and mature eggs.

BREVILEGNIA UNISPERMA var. LITORALIS

Figs. 10–13. Irregular oogonia, common types.

Figs. 1 and 13 × 347; figs. 2, 5–8 and 10–12 × 600; fig. 3 × 95; fig. 4 × 143; fig. 9 × 876

PLATE 31

BREVILEGNIA UNISPERMA var. DELICA

Fig. 1. Young sporangium.
Fig. 2. Sporangium showing spore initials.
Fig. 3. Sporangium and young oogonia.
Fig. 4. Typical habit of sporangia in rather old culture. These sporangia are thicker and shorter than the average.
Fig. 5. Sporangium of type intermediate between *Dictyuchus* and *Thraustotheca*. Spores in position and sprouting as in *Dictyuchus* but sporangial wall disintegrating as in *Thraustotheca*.
Fig. 6. Long sporangia beginning to liberate spores.
Fig. 7. Sporangium with wall entirely gone and spores spreading.
Fig. 8. Habit of oogonia.
Figs. 9 and 10. Oogonia showing mature egg structure.

DICTYUCHUS sp. ?

(See this Journal **42**: 144, pl. 15. 1926)

Fig. 11. Sporangium to show the retention of the sporangial wall.
Fig. 12. Sporangium showing the usual *Dictyuchus* network after the spores emerge. The spores do not separate as in *Brevilegnia*.

Figs. 1–3, 5, 6 × 213; fig. 4 × 90; figs. 7, 8 × 383; figs. 9, 10 × 934; figs. 11, 12 × 144

PLATE 32

BREVILEGNIA LINEARIS

Figs. 1, 2. Oogonia and antheridia in various stages, showing habit.
Figs. 3–5. Sporangia after the wall has disappeared; spores still hanging together and of various sizes.
Figs. 6, 7. Younger sporangia, showing wall intact.
Figs. 8, 9. Oogonia with antheridia, one showing a ripe egg.

Fig. 10. Oogonium with three antheridia, a rare case. For some unknown reason the oogonium in this case was empty when found.
Figs. 11, 12. Spores sprouting and still in large part held together.

Figs. 1, 2, 7, 11, 12 × 347; figs. 3, 6 × 239; figs. 4, 5 × 144; figs. 8–10 × 600

PLATE 33

LEPTOLEGNIA ECCENTRICA

Fig. 1. Branched sporangium. × 437.
Fig. 2. Section of sporangium showing elongated spores. × 437.
Figs. 3–4. Typical sporangia. × 437.
Figs. 5–7. Young oogonia with androgynous antheridia. × 647.
Fig. 8. Oogonium with mature egg and androgynous antheridium. × 647.
Figs. 9–10. Oogonia with mature eggs. × 647.

PLATE 34

APLANES TRELEASEANUS

Fig. 1. Long intercalary oogonium. × 222.
Fig. 2. Oogonium with a long spike at apical end. × 222.
Fig. 3. Two oogonia in a chain. × 222.
Fig. 4. Oogonium with androgynous antheridia terminating a chain of gemmae. × 222.
Fig. 5. Large oogonium with androgynous antheridia. × 222.
Fig. 6. Small oogonium with one egg. × 222.
Fig. 7. Oogonium borne on a lateral branch. × 222.
Fig. 8. Smooth oogonium showing conspicuous pits. × 222.
Fig. 9. Mature egg with cup of oil drops at both ends. × 437.
Fig. 10. Typical mature egg. × 437.
Fig. 11. Sessile oogonium with many short papillae. × 222.

PLATE 35

APLANES TRELEASEANUS

Fig. 1. Chains of gemmae. × 58.
Fig. 2. Chain of gemmae showing characteristic small branches. × 58.
Fig. 3. Chain of gemmae showing relation of sporangia to them. × 58.
Fig. 4. Small dictiosporangium. × 222.
Fig. 5. Sporangium showing empty cysts at the mouth and part of the spores encysted in the sporangium. × 222.
Fig. 6. Spores in a single row formed in a chain of gemmae. × 222.
Fig. 7. Spores sprouting at the mouth of the sporangium. × 222.
Fig. 8. Typical sporangium with spores sprouting *in situ*. × 222.
Fig. 9. Clump of spores which had escaped from the sporangium and sprouted without a second swimming stage. × 222.

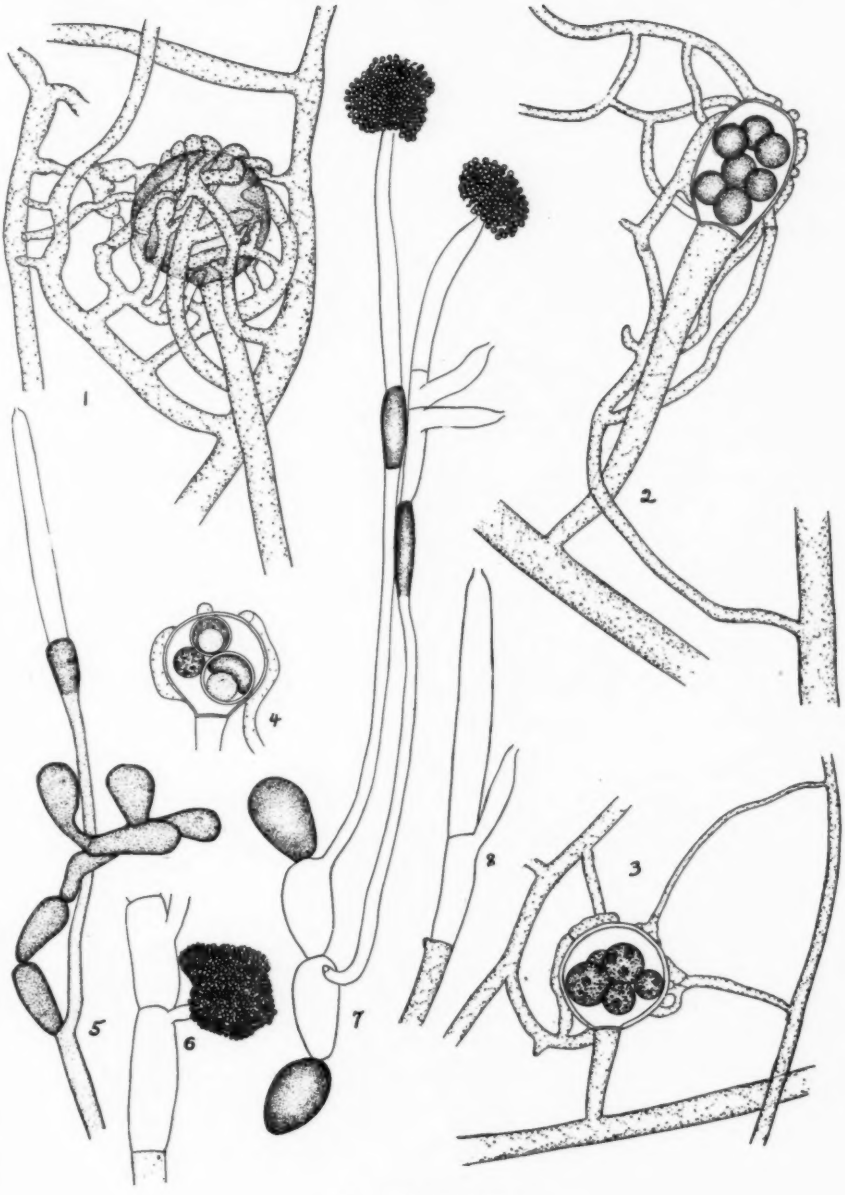
PLATE 36

DICTYUCHUS ACHLYOIDES

Fig. 1. Sporangium of *Dictyuchus* type.
Fig. 2. Sporangium of *Achlya* type.
Fig. 3. Sporangium of *Achlya* type with oogonia and antheridia below.
Fig. 4. Sporangium of *Dictyuchus* type with oogonium and antheridia.
Fig. 5. Long sporangium of *Dictyuchus* type.
Figs. 6 and 7. Small sporangia.
Figs. 8–10. Oogonia, young and old.
Fig. 11. Oogonial initial which has sprouted to form a sporangium.

Figs. 1–7 and 9, 11 × 213; figs. 8 and 10 × 383

PLATE 27



ACHLYA BISEXUALIS

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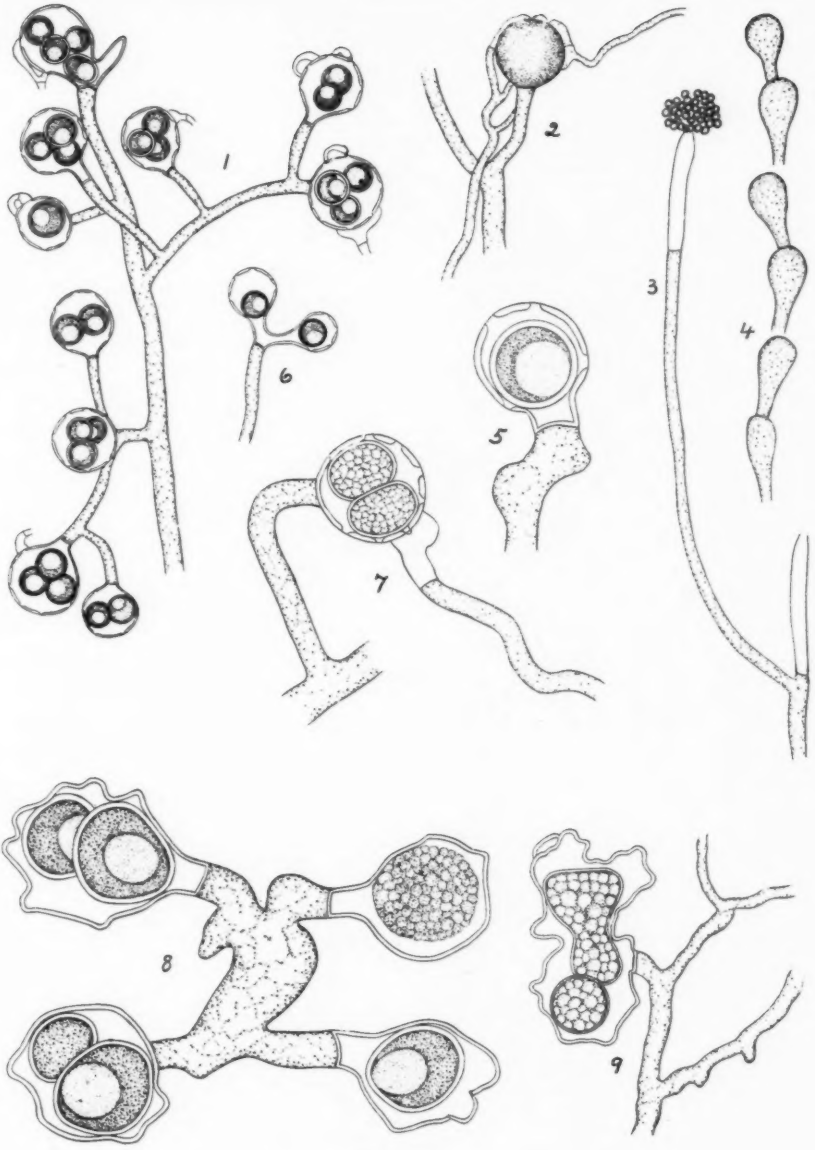
PLATE 28



ACHLYA ABORTIVA FORMA NORMALIS, FIGS. 1–7
ACHLYA ABORTIVA, CONTORTED FORM, FIGS. 8 AND 9

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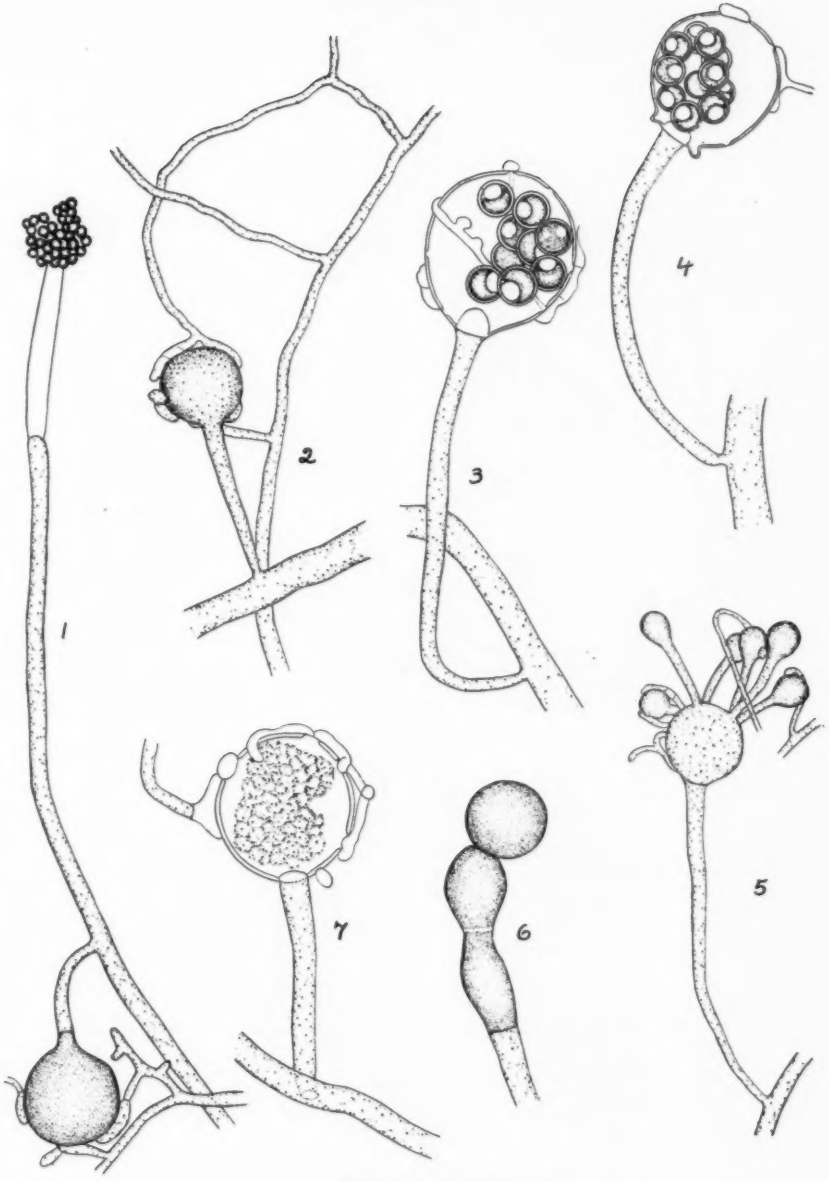
PLATE 29



ACHLYA INFLATA

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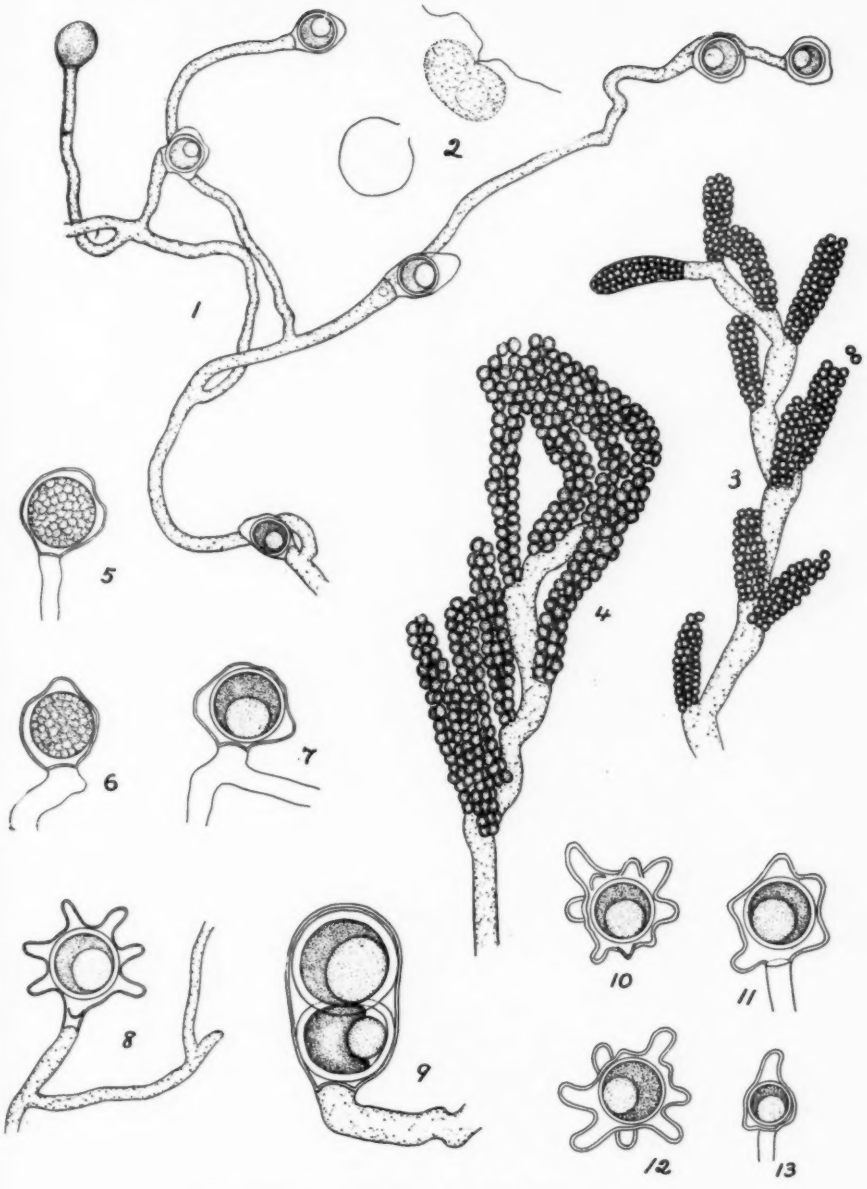
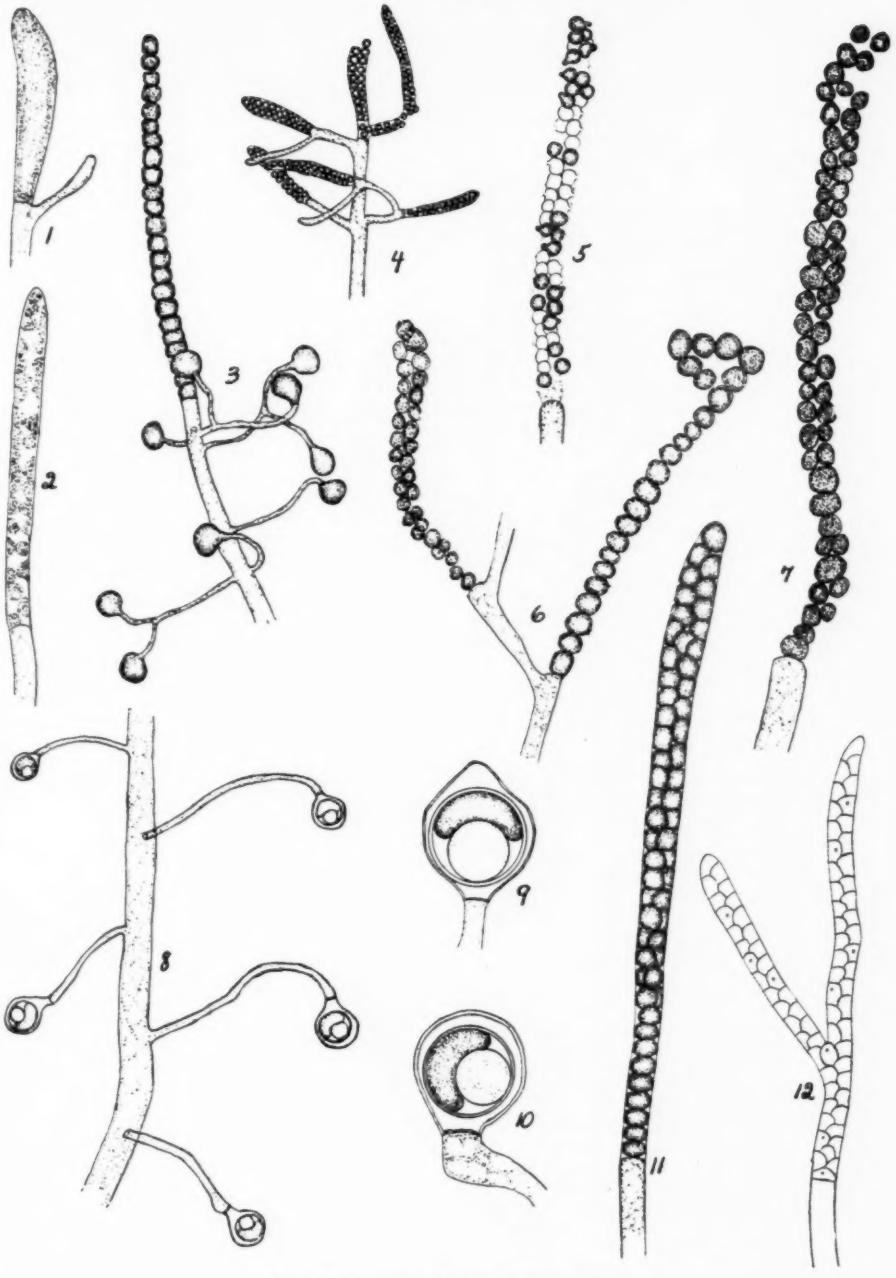
PLATE 30



BREVILEGNIA UNISPERMA VAR. MONTANA, FIGS. 1-9
BREVILEGNIA UNISPERMA VAR. LITORALIS, FIGS. 10–13

PLATE 31



BREVILEGNIA UNISPERMA VAR. DELICA

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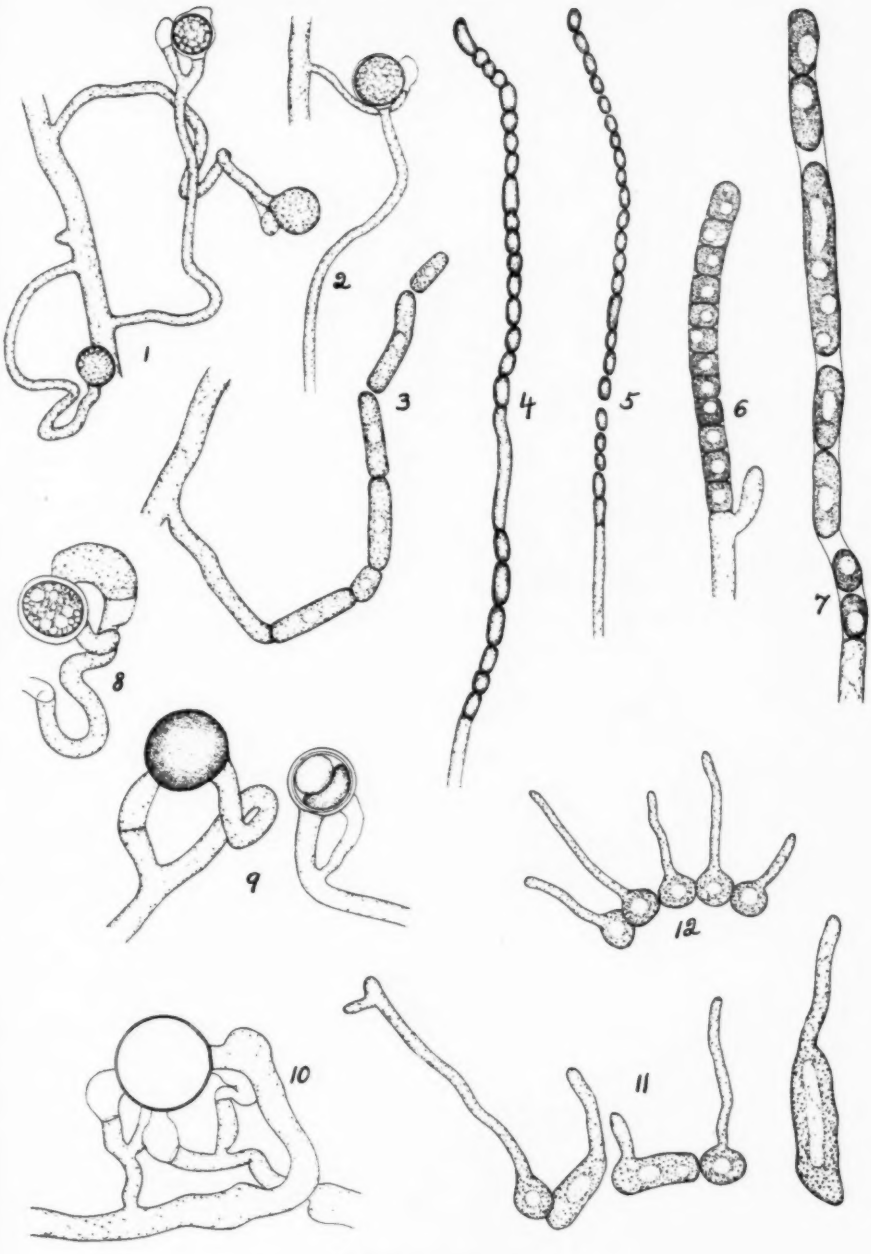
PLATE 32



BREVILEGNIA LINEARIS

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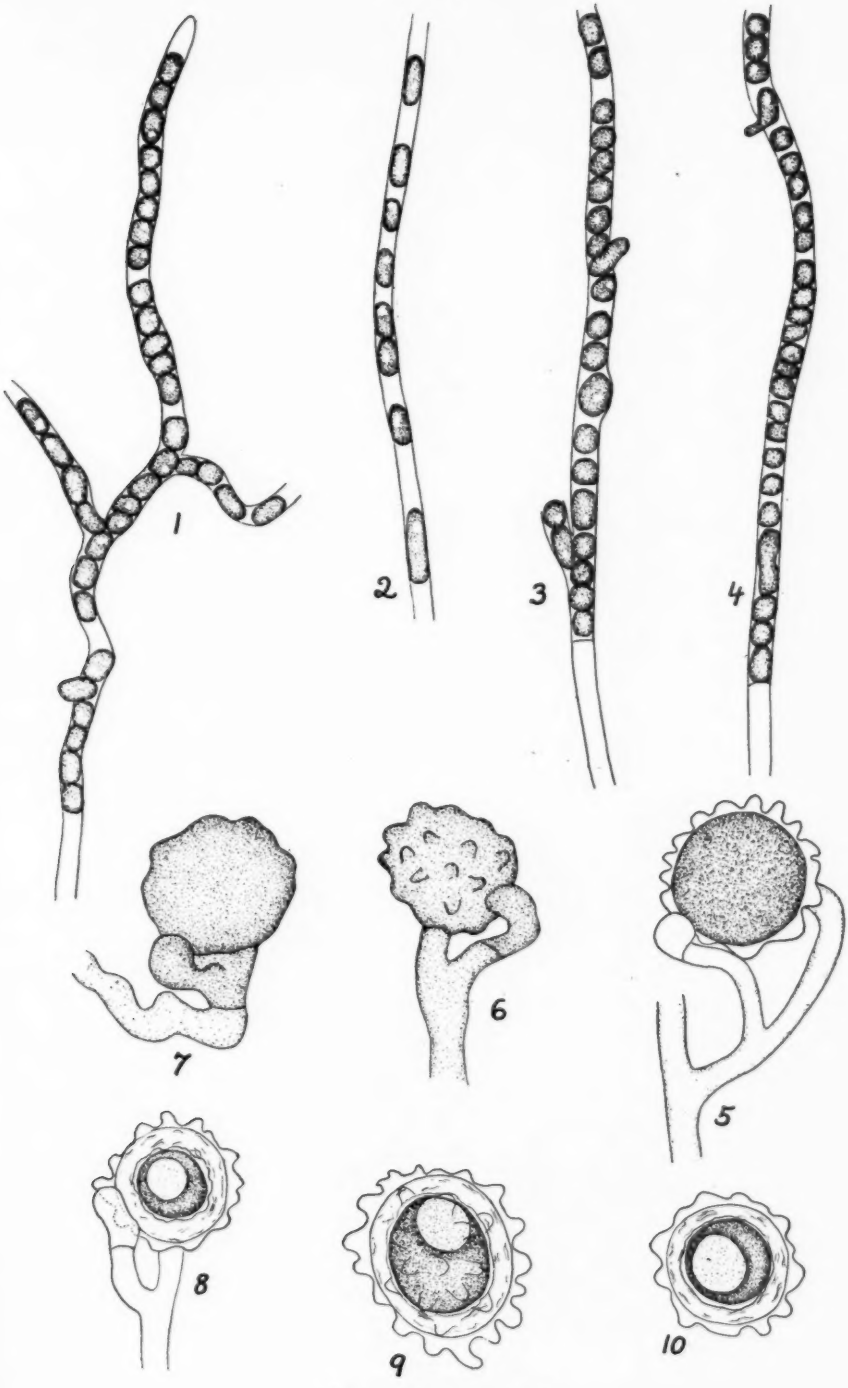
PLATE 33



LEPTOLEGNIA ECCENTRICA

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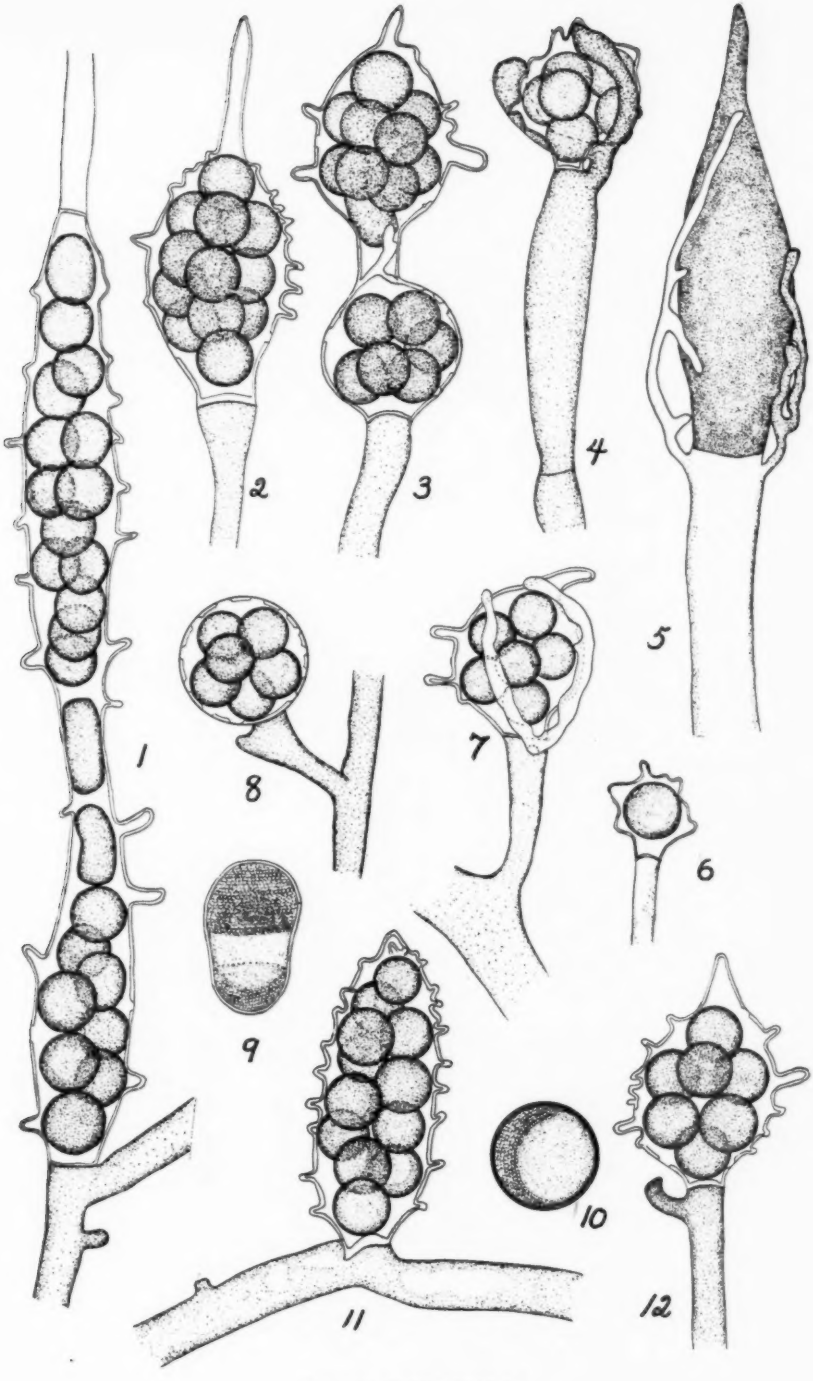
PLATE 34



APLANES TRELEASEANUS

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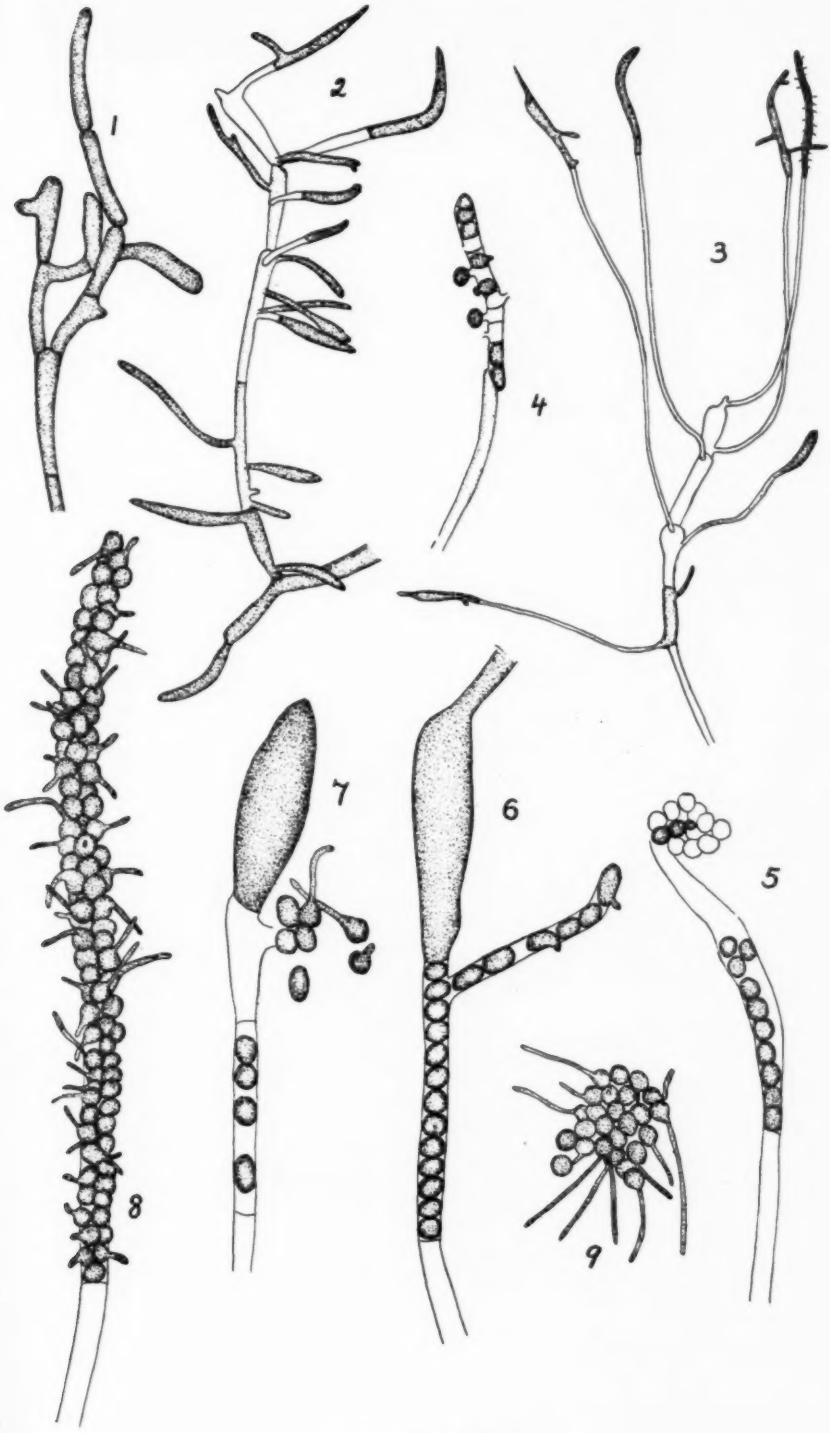
PLATE 35



APLANES TRELEASEANUS

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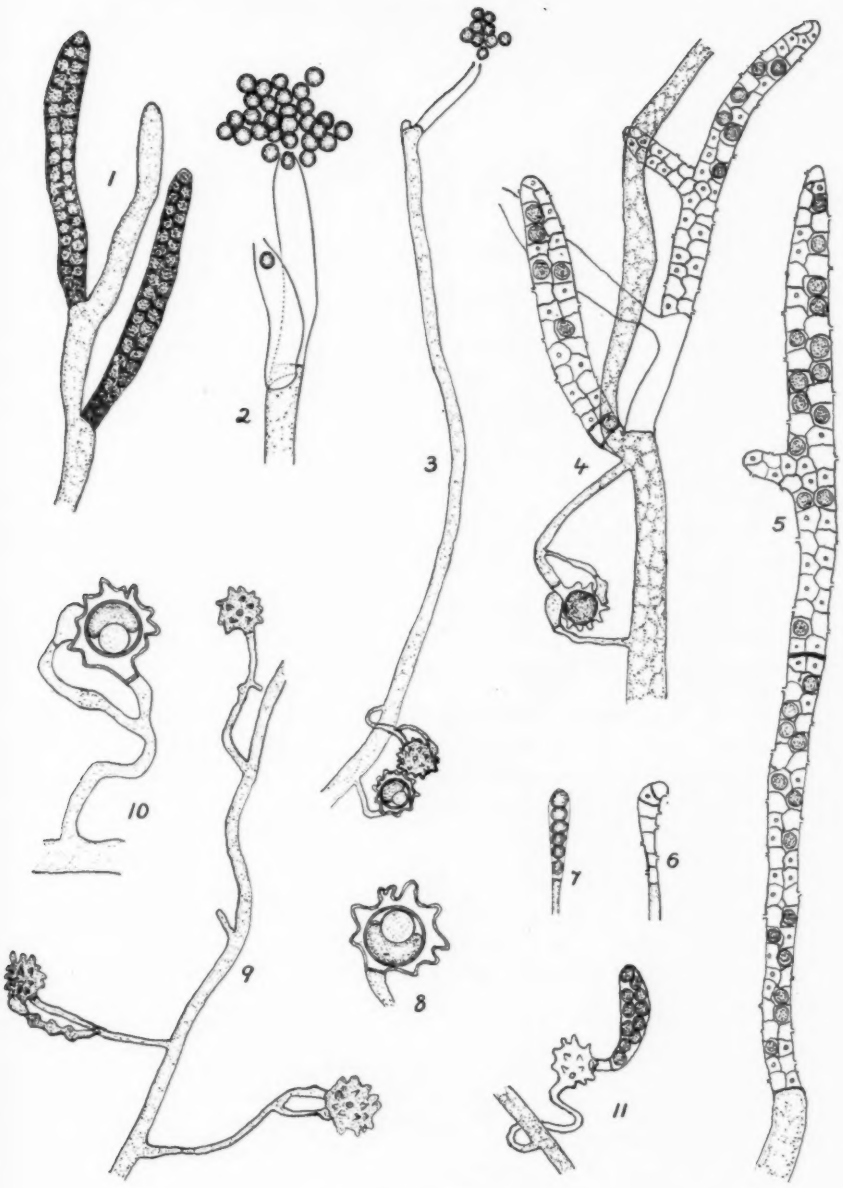
PLATE 36



DICTYUCHUS ACHLYOIDES

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SOME NEW WATER FUNGI FROM THE SOIL, WITH OBSERVATIONS ON SPORE FORMATION

By J. N. Couch[1]

Plates 37–43

In the present paper the writer presents some of the more important results obtained in studies on the water molds collected from the soil around Cold Spring Harbor, New York. Both of the species of *Geolegnia* described by Coker and Harvey ('25) from Chapel Hill, N. C., have been found in the soil on Long Island, *G. inflata* having been found once and *G. septisporangia* several times. Two new species of *Brevilegnia* have also been collected from the soil.

Methods of Collecting and Purifying

The method of collecting the molds was as follows: Amounts of soil each about equal to a heaping tablespoonful were collected in small wide mouth bottles. The soil was then poured into large Petri dishes and enough water was added to the dish to completely cover the soil. After the water had become clear, bits of boiled hemp seed were added. The dishes were then covered and allowed to stand for several days. After four or five days fungus growth had occurred on almost all the seed. Only very rarely, however, was this growth that of a single one of the *Saprolegniaceae*. Usually other members of the family were present and quite often one of the *Mucorales* or an *Ascomycete* appeared. Bacteria and numerous protozoan organisms were usually present in great abundance.

The hemp seed with its fungus growth was transferred to a clean, sterile dish and washed with a strong stream of water from a wash

[1] National Research Fellow.

This work was done in Dr. A. F. Blakeslee's laboratory at Cold Spring Harbor, L. I., N. Y., and in Dr. B. M. Duggar's laboratory at The Missouri Botanical Garden, to both of whom the writer wishes to extend his thanks for the facilities of their laboratories and for suggestions during the progress of the work. The writer also wishes to thank Dr. W. C. Coker for suggestions throughout the work.

bottle to remove the particles of trash, earth, and as many of the free floating organisms as possible. A microscopic examination showed which of the fungus organisms, if any, were worth isolating. Much of the mycelium of undesirable fungi was removed from the fungus to be isolated with fine needles under a dissecting, binocular microscope.

A single thread of the desired fungus with a sporangium at its tip about ready to discharge spores was cut from the culture with a needle sharpened so that it had a chiseled edge. This operation was carried out under the binocular. The thread was transferred to a drop of sterile water on a flamed slide. After the spores emerged, the thread was removed, and the spores were stirred through the drop. A small drop of the spore suspension was picked up with a platinum loop and this drop was gently spread in a line over a corn meal agar plate. The line made by the water was marked with a wax pencil on the lower surface of the Petri dish to facilitate the finding of the spores when they began to sprout. When the spores had begun to sprout, several were picked up one by one with a little agar under the binocular, and transferred to fresh corn meal agar plates. For obtaining single spore cultures this method, of several tried, has proved the most useful to the writer.

DESCRIPTION OF SPECIES

Brevilegnia bispora n. sp.

Plates 37 and 38

Mycelium forming a dense growth on hemp seed; reaching a diameter of 3 cm. Hyphae up to 1.5 cm. long, not rarely as much as 65μ thick near the base; considerably branched. Sporangia very abundant, 21–45 $\times$ 125–400μ, terminal, renewed by cymose branching as in *Achlya*, long clavate, broadest near the middle. First sporangia resembling the *Achlya* type, many of which, however, lack a distinct papilla of dehiscence, a considerable part of the sporangial tip giving away for the exit of the spores; later sporangia of the same shape and size as the early ones but dehiscing by the swelling of the spores and consequent bursting of the sporangial wall much as in the later sporangia of *Thraustotheca primoachlya;* more numerous than the first type. Spores of the *Achlya* type mostly 10.8μ thick and spherical when encysted; spores of the *Thraustotheca* type up to 18μ thick and polyhedral when encysted, usually with a large conspicuous vacuole; spores of the *Achlya* type usually diplanetic but those of the *Thraustotheca* type remaining inactive or sprouting *in situ* if fresh water is added. Gemmae formed in considerable numbers and especially under unfavorable conditions as when the cultures become old or infected with bacteria,

spherical, pyriform, or cylindrical, often in chains. Oogonia usually very abundant, but appearing only after the cultures are three or more days old; very small, 16–28.8μ thick, most about 21.6μ thick, spherical, smooth, formed usually singly but sometimes in pairs on the ends of very long, delicate stalks which arise from the main branches. Eggs 11.3–19.5μ, most about 18μ thick, spherical, eccentric when ripe. Antheridia one to several on most of the oogonia, of androgynous origin, often arising from the oogonial stalk itself. Antheridial tube developed.

This remarkable plant has appeared but once and then it was found in a soil collection in deciduous woods near Cold Spring Harbor, L. I., May 5, 1926.

In the behavior of its sporangia and spores this species shows affinities to both *Achlya* and *Thraustotheca*, resembling most closely *T. primoachlya*, which also combines the sporangial characters of both genera. The *Achlya*-like sporangia, with their fragile and quickly disappearing walls, could hardly be distinguished from the same type of sporangia in *T. primoachlya* but the *Thraustotheca* type of sporangia show a considerably wider variation than is found in *T. primoachlya*. In a good many of the later formed sporangia the spores are quite large and show a superficial resemblance to *Geolegnia*. That this resemblance is only superficial, however, is proved by the fact that the contents of these large spores will sometimes divide forming several normal motile zoospores.

Brevilegnia subclavata n. sp.

Plates 39–41 and 42, figs. 1–7

Mycelium forming a rather dense growth on hemp seed; reaching a maximum diameter of 0.9 cm. Hyphae short, usually not more than 3 mm. long; up to 62μ thick at the base; considerably branched. Sporangia formed in great abundance, 30–100 $\times$ 108–140μ, terminal, the renewal of the sporangia taking place by cymose branching as in *Achlya* or *Thraustotheca* and also by successive formation in basipetal series, as many as twelve sporangia sometimes being formed in a single row; usually short clavate, but varying from nearly spherical to long cylindrical with spores in a single row. Sporangia without any papillae of dehiscence, the spores escaping by swelling and bursting the sporangial wall, or not rarely remaining within the sporangium and sprouting *in situ*. Spores 10.8–19 $\times$ 10.8–28.8μ, varying from nearly spherical to cylindrical, but usually distinctly polyhedral in shape, with a distinct large vacuole; without a swimming stage. Sexual reproduction occurring in most but not in all cultures, appearing after the culture is three or more days old. Oogonia 19–25μ, spherical, but more often slightly subspherical, formed on the ends of very delicate, usually long,

stalks which arise racemosely from the much thicker, main branches. Eggs 15–19μ thick, spherical, single in the oogonium, eccentric. Antheridia on most oogonia, androgynous, usually arising from the oogonial stalk itself; becoming practically empty during the early development of the egg.

This peculiar species has appeared once in numerous soil collections from Long Island and then from soil taken from the hillside covered with deciduous trees east of the Eugenics Record Office, at Cold Spring Harbor, April 1, 1926.

The shape of the sporangia, the way in which they are borne, and their general manner of dehiscence, by the swelling of the spores and the consequent bursting of the sporangial wall, show a striking resemblance to the genus *Thraustotheca*, especially the species *T. clavata*. In the method of spore formation and in the shape, size and structure (uninucleate character) of the commonest type of spore the fungus also resembles *Thraustotheca*. On the other hand the vegetative growth, the non-motility of the spores, the numerous compound spores formed in all cultures, and the small oogonia containing a single eccentric egg all suggest an affinity to *Geolegnia*.

This species may be distinguished from *Brevilegnia unisperma* Coker and Braxton and the variety *litoralis* by the shape of the sporangia and the manner of dehiscence and by the fact that the spores are motile in the two plants described by Coker and Braxton and nonmotile in the present species. The plants may also be separated on oogonial characters, the walls of the oogonia of *B. subclavata* being much more nearly smooth than in *B. unisperma* and its variety.

The sporangia of *Brevilegnia diclina* Harvey (described in this issue of the Journal), the development of which the writer has studied both on living material and by killing and staining, show affinities both to *Thraustotheca* and *Geolegnia*. The spore initials in the commonest type of sporangium are uninucleate as are also the ripe spores, but quite often in old cultures sporangia which are almost precisely like those of *Geolegnia septisporangia* are also formed. These latter contain large compound multinucleate spores formed in a single row. The spores, moreover, in this species are nonmotile under ordinary conditions of culture but motility can be induced by special treatment (see below). The non-motile character of the spores and the compound spores relate the plant to *Geolegnia* while the power of motility, normally latent, suggest, along with the other sporangial characters, a kinship to *Thraustotheca*. The species resembles *Geolegnia* in sexual reproduction.

This plant, *B. diclina*, can easily be distinguished from the present species, *B. subclavata*, by sporangial characters alone; for in the first species the sporangia are long cylindrical while in the latter they are short clavate.

SPORANGIAL DEVELOPMENT

The sporangial development of *Brevilegnia subclavata*, *Geolegnia septisporangia* and *G. inflata* has been studied in living material and by killing and staining.

For killing and staining material the method used was essentially as follows. Material was fixed directly in the culture dish in 0.5 per cent chromic, 1 per cent acetic acid for about twenty-four hours, after draining off the culture water. The fixed material was then poured into a large dish containing water and allowed to wash for twenty-four hours, the water being frequently changed. Alcohol 15, 25, 35, 50 and 70 per cent two hours each. Water 5 min. Threads were then cut off and placed in a small drop of water on a slide smeared with a thin coating of albumen fixative. The surplus water was drained off the slide with a small piece of filter paper and the remaining water was evaporated, with gentle heat, until the preparation was almost dry. This procedure dried some of the threads around the margin of the preparation thus fixing them to the slide. Great care had to be taken not to let the material dry too much. Material was then stained in an aniline solution of Coleman and Bell's improved gentian violet for 5–10 min. Water 1 min. Potassium iodide solution 3 min. Alcohol 80 per cent 30 sec.; 95 per cent 1 min.; 100 per cent 1 min. Clove oil 30 sec.; cedar oil 30 sec. Xylol 3 min. Mount in balsam. The writer has tried several shorter methods but none gave good results.

BREVILEGNIA SUBCLAVATA

In the commonest type of sporangium, the clavate form, the development of the sporangium and its manner of dehiscence is very much like that described by Weston ('18) for *Thraustotheca clavata*.

In this species the primary and commonest type of sporangia is formed on the tips of the main hyphae. The hypha then sprouts just below the base of the sporangial wall, grows for a short distance and then forms another sporangium. This process may be repeated several times until the hypha comes to have a zigzag appearance. Secondary sporangia may also be formed in basipetal series.

A thread which is going to form a sporangium becomes densely filled with protoplasm near its tip with a proportionate enlargement (pl. 41, fig. 1). The young sporangium continues to enlarge until it is from two to four times as thick as the diameter of the thread which bears it (fig. 2). In the center of the young sporangium and extending for some distance down the thread is a large central vacuole (figs. 1, 2 and 3). As development proceeds the protoplasm passes up into the sporangium, filling the distal part and pushing back the vacuole (figs. 2 and 3). The protoplasm bordering the vacuole near the base of the sporangium takes on a greenish color and shortly in this region the basal wall is laid down (figs. 3 and 4). Meanwhile the vacuole has become longer and narrower and has begun to push out toward the lateral walls of the sporangium cutting the contents up into the spore origins (fig. 5).

This cleavage process continues until the protoplasmic contents have been cut up into numerous spore initials (figs. 6 and 7). Shortly after the spore initials have become distinct as individuals their outlines suddenly run together until the clefts which separated them have completely disappeared (figs. 8 and 9). This apparent fusion of the spore initials begins from the outside and passes toward the center of the sporangium so that the outer margins of the spore initials will have completely disappeared while the inner margins are still distinct (fig. 8). Following immediately upon the complete disappearance of the spore initials, numerous small vacuoles appear which grow in size and then burst (fig. 9). Within a very few minutes after the spore initials have disappeared completely, the final spores begin to appear as individuals (fig. 10).

The spores now take on as nearly a spherical shape as their mutual pressure against each other and against the sporangial wall will permit. This pressure is usually so great that the spores assume a distinctly polygonal shape. The spores now swell pushing against each other and against the wall with such force that the contents of the sporangium appear almost homogeneous (fig. 11). This swelling continues until the sporangial wall bursts and part of the spores are expelled with considerable force. The sporangial wall usually bursts at the tip, though it may burst anywhere on the side, and the number of spores shot out in this initial expulsion may vary from two or three to half the entire number originally in the sporangium. The spores left in the sporangium, after this initial expulsion, continue to swell and those near the opening are pushed out. A few spores, however, are invariably left in the sporangium. The discharged spores and those left within

the sporangium now encyst. The shape of the encysted spore is subspherical to polygonal. Under proper conditions the spores will germinate almost immediately after being formed.

As the culture gets older or becomes infected with bacteria large compound spores are formed in the sporangia. The development of one of these sporangia is shown on plate 42 (figs. 1 and 7). The early stages—the segmentation of the sporangial contents into numerous spore initials and the disappearance stage—are the same as have been described above, but the development from the disappearance stage on is quite different from the type described above. Instead of the final spores appearing in only a few minutes after the disappearance stage in approximately the same numbers as there were spore origins, the development here is very much retarded. The disappearance stage lasts from thirty minutes to an hour. The contents of the sporangium shown on plate 42, figure 4, became constricted into two parts, the line of cleavage beginning from the outside and passing inwards, just as in *Geolegnia septisporangia*. Other lines of cleavage started but were not completed (fig. 4). Simultaneously there appeared numerous small vacuoles. These grew in size and some fused. At the same time the two large protoplasmic bodies in the sporangium, which may be designated compound spores, swelled considerably, as was shown by the outward bulging of the end walls of the sporangium, which, during the disappearance stage, were turned inward. This swelling continued until the wall of the sporangium burst and one of the spores popped out.

The mycelium and sporangia are multinucleate. The structure of the nucleus in the mycelium and in the young sporangium is the same as has been described by Davis ('03), Claussen ('08), myself ('24) and others. It was found that the spore initials were almost invariably uninucleate (pl. 41, fig. 13) as were also the fully formed spores (fig. 14) in sporangia of the *Thraustotheca* type. In sporangia of the *Geolegnia* type, however, the spores were multinucleate.

GEOLEGNIA INFLATA

Plate 43

In *Geolegnia inflata* the primary sporangia are formed on the ends of the larger hyphae. These soon become inflated at regular intervals (pl. 43, fig. 1). The number of inflations corresponds to the number of spores formed in a sporangium and these may vary from one to fifteen. The protoplasm becomes considerably denser in the inflations than in the

rest of the hypha. The sporangium is multinucleate, the nuclei being located in the inflations, though occasionally one may be seen between swelling (pl. 43, fig. 14). As few as four nuclei have been counted in an inflation and as many as eight. A basal septum is formed cutting off the sporangium from the rest of the hypha. This septum is at first straight but soon becomes convex outwards with relation to the sporangium. The position of the septum depends upon the relative turgidity of the sporangium and hypha.

After the basal wall is formed the central vacuole pushes slowly outwards cutting the protoplasm up into numerous small masses (pl. 43, figs. 1 and 7). These masses are doubtless homologous to the spore initials in *Saprolegnia* and *Achlya*, and are designated as such here. The number of spore initials in each inflation may vary from two to seven. So far the writer has been unable to get good stained preparations showing the nuclear condition of the sporangium at this stage. From the nuclear condition in the young sporangium and the mature spores it would seem certain that the spore initials contain from one to four nuclei.

The membrane lining the wall is thinned down between the spore initials until it breaks. The spore initials then swell and fuse until the contents of the sporangium become almost homogeneous (pl. 43, figs. 2 and 8). This homogeneous stage lasts only about a couple of minutes. The protoplasm then slowly shrinks from the wall and passes toward the center of the inflation. At this time denser regions can often be seen in the protoplasm corresponding to the masses before the disappearance stage (pl. 43, figs. 3, 9 and 10).

During this process the protoplasm exhibits considerable movement. In the figures 1–5 shown on plate 43 attention was focused on the contents of the two upper inflations and especially on the narrow region joining them. When the spore initials began swelling, the initial at "a" began moving down through the neck toward the second inflation. Meanwhile the membrane lining the narrow region shrank from the wall. The spore initial at "a" fig. 3 was apparently pulled down into the second inflation and then was immediately pulled back into the first inflation by the apparent contraction of the protoplasm. During this process the almost hyaline protoplasm connecting the more granular protoplasm in the inflations seemed to exhibit elastic properties much as an elastic rubber band.

As this contraction goes on numerous vacuoles are formed in the surface and these ultimately burst (figs. 5 and 6). The protoplasmic strand

connecting the large spores becomes thinned down until it is pulled in two and then the ends are drawn into the spores. The spores now assume a round or oval shape depending upon the size and shape of the inflation and form a thick wall around themselves.

The mature spore is multinucleate, the number of nuclei corresponding closely to the number of nuclei in an inflation and to the number of spore initials formed before the disappearance stage (figs. 13 and 14).

GEOLEGNIA SEPTISPORANGIA

Plate 42, figs. 8–23

In this species the primary sporangia are formed on the ends of the main hyphae and secondary sporangia are formed in basipetal series beneath. The sporangia are long cylindrical to clavate and the number of spores vary from one to fifteen.

The development of a sporangium is shown on plate 42, figures 8 to 18. This one was kept under observation for over three hours, from an early stage before the formation of the basal wall to the formation of the mature spores.

The sporangium shown in figure 8 contained a dense peripheral layer of protoplasm and a long, conspicuous, central vacuole which extended back down into the thread. On the tip of this sporangium was a shiny thickened cap, the basal wall of an older sporangium formed in the same thread. Protoplasm passed into the sporangium in the form of minute granules which apparently passed along delicate hyaline threads or in the form of large masses of granular protoplasm. This movement continued until all but a small remnant of the once long and conspicuous central vacuole was obliterated (figs. 9 and 10). A basal wall was now formed separating the sporangium from the hypha. This was at first straight (fig. 11) but later (about one hour) became concave (fig. 12). Meanwhile many small vacuoles had formed near the center.

Twenty-five minutes later the small vacuoles had disappeared and an irregularly oval-shaped vacuole was partially surrounding a large, dense, central mass of protoplasm. The basal wall had changed from concave to convex. This change in the position of the basal wall was not due, however, to the rupture of the membrane lining the sporangial wall, for this was still intact, the rupture taking place thirty minutes later, but was caused by the increased turgidity of the hyphal thread below the sporangium. This turgidity of the hyphal thread was so great that not only was the basal wall pushed up into the sporangium but the

lateral wall of the sporangium was pushed in near the base by the outward bulging of the hyphal wall (fig. 14).

The contents of the sporangium was finally divided into two large masses of protoplasm eccentrically joined to the peripheral lining-membrane (fig. 15). These large masses, which may be regarded as the initials of the compound spores resemble, except for their larger size, fewer number, and multinucleate character, the spore initials in *Saprolegnia* and *Achlya*. As in the other species of *Geolegnia* (*G. inflata*), the lining membrane is thinned down until it breaks and the spore initials then swell until the lines separating them have disappeared (fig. 16). This stage corresponds to the disappearance stage in *Saprolegnia* and *Achlya*.

About three minutes after the outlines of the spore initials had completely disappeared, the final spores began to contract and round up (figs. 17 and 18). As this goes on numerous small vacuoles come to the surface and burst. In twelve minutes after the contraction process began the spores had attained their final shape.

In *G. septisporangia* the number of spores formed almost invariably corresponds to the number of spore initials. Exceptions to this have been seen, however, as when five spore initials were formed and only three spores.

The nuclear behavior during spore formation in this species shows a very unique condition not encountered heretofore in the water fungi. The killing and staining methods used here have been described earlier in the paper.

The young sporangium such as is illustrated on plate 42, figures 8, 9, and 10 is multinucleate and the nuclei are distributed with more or less regularity throughout the protoplasm. As the spore initials are formed, the nuclei become massed in the center of what is to become a spore initial. Figure 19, showing the killed and stained condition, corresponds approximately to figure 14 showing the living condition. The nuclei in each spore origin were imbedded in a very dense and deeply staining cytoplasm and in some cases were so closely pressed together as to appear that they were fusing. As few as five nuclei have been counted in the spore origin and as many as twelve. The nuclei remain closely pressed together imbedded in the dense region of cytoplasm until after the spores are finally formed. They then separate from each other and become dispersed more or less evenly through the spore (figs. 20–23).

EXPERIMENTS TO INDUCE MOTILITY IN THE SPORES

Numerous experiments have been carried out to induce the formation of motile spores in *Brevilegnia subclavata*, *Geolegnia inflata* and *G. septisporangia* but so far without success. Experiments are still under way and therefore the writer is not making a detailed report at present.

Experiments have also been carried out with *B. diclina* Harvey[2] (described elsewhere in this issue). This species as grown in ordinary distilled or tap water has never been observed to produce motile spores. After considerable experimentation the writer succeeded in inducing the formation of motile spores in two ways.

In the first case motile spores were formed when the fungus was grown on a piece of boiled hemp seed in distilled water, the pH value of the water having been adjusted to about pH 4 by the addition of 2 cc. of 0.01 N HCl to 250 cc. of distilled water. Several other solutions of water were used with pH values varying from 3 to 8 but only in the cultures with the pH value about 4 were motile spores formed. In these solutions the motile spores were formed only in very small numbers and only during the first 48 hours of growth. In some cases spore motility was induced in old cultures by transferring them to distilled water with a pH 4 value, the motile spores being formed about eight hours after the transfer.

In the second case motile spores were formed in great abundance when the distilled water in which the cultures were grown was first treated with animal charcoal. Twenty grams of animal charcoal were put in 1000 cc. distilled water and then filtered and autoclaved. The distilled water had a pH value of about 5.8–6 before treatment with animal charcoal, but after treatment the pH value was raised to about 6.6–6.8. Neither filtering nor autoclaving had any appreciable effect on the pH value. If the water treated with animal charcoal is redistilled and cultures are then grown in this water, no motile spores are formed.

An explanation of these peculiar and apparently conflicting results—where in the one case motile spores were formed by lowering the pH and in the other were formed when the water was treated with animal charcoal which raised the pH value—will have to be deferred until further experimentation.

The motile spores emerged from the encysted state and swam in the laterally biciliate form as is characteristic in the genus *Thraustotheca*.

[2] The writer was given a culture of this fungus by Dr. Coker who obtained his material from Mr. Harvey.

OTHER WATER FUNGI FROM THE SOIL

The following were collected from soil near Cold Spring Harbor L. I., N. Y., during the late winter and spring of 1926.

Thraustotheca clavata, several times
Achlya glomerata, several times
Achlya caroliniana, several times
Achlya imperfecta, once
Aphanomyces scaber, once
Leptolegnia subterranea, several times
Allomyces arbuscula, several times
Pythium (several species), common

The following were collected from the soil in the Blue Mountains at Abbey Green, Jamaica, B. W. I., at an elevation of 3500 feet during the summer of 1926.

Thraustotheca clavata, several times
Achlya caroliniana, several times
Achlya imperfecta, once
Achlya sp., once

SUMMARY

Two new species of *Brevilegnia* are described.

The spore development in *B. subclavata* and in *Geolegnia inflata*, and *G. septisporangia* has been studied. In all three species spore initials are formed which disappear completely before the final spores are formed. In *B. subclavata* the spore initials in the commonest type of sporangium are uninucleate as are also the final spores. In this species compound, multinucleate spores are also formed. In *G. inflata* spore initials are formed some of which may be uninucleate, others of which may contain two or more nuclei. These spore initials fuse to form a multinucleate spore. In *G. septisporangia* the spore initials are multinucleate, the nuclei grouping together in a peculiar fashion as though preparing to fuse. The mature spore is also multinucleate and the number of spore initials usually corresponds in number to the number of spores finally formed, though sometimes the number of spore initials may be greater.

Numerous experiments have been carried out to induce motility in the spores of *Brevilegnia subclavata* and the two species of *Geolegnia*, but so far without success. In *Brevilegnia diclina* motile spores have been formed when cultures were grown on boiled hemp seed in distilled

water with a pH adjusted to about 4; and also, and in greater numbers, when the distilled water was treated with animal charcoal.

CHAPEL HILL, N. C.

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EXPLANATION OF PLATES

PLATE 37

BREVILEGNIA BISPORA

Fig. 1. Photograph of part of a culture on a hemp seed showing rather depauperate mycelium. × 9.
Fig. 2. Microphotograph showing sporangia and oogonia. × 80.
Fig. 3. Sporangia, most of the *Thraustotheca primoachlya* type. × 80.
Fig. 4. Sporangia and oogonia. × 80.
Fig. 5. Sporangium approaching the *Geolegnia* type, spores large with conspicuous vacuole. Note oogonia. × 200.
Fig. 6. Oogonium with ripe eccentric egg and androgynous antheridium arising from oogonial stalk just beneath the oogonium. × 580.

PLATE 38

BREVILEGNIA BISPORA

Figs. 1–5. Various types of sporangia. × 207.
Fig. 6. Spore emerging from cyst. × 834.
Fig. 7. Gemmae. × 140.
Fig. 8. Habit of oogonia, antheridia, and sporangium. × 207.
Figs. 9, 10. Oogonia and antheridia. Note the size of the egg in figure 9 as compared with the spores. × 573.

PLATE 39

BREVILEGNIA SUBCLAVATA

Fig. 1. Photograph of a culture on hemp seed, showing reduced mycelium and sporangia. × 9.
Fig. 2. Sporangia of the *Thraustotheca* type. × 200.
Fig. 3. Sporangia in various stages of development, of the *Thraustotheca* type. × 80.
Fig. 4. Sporangia of the *Thraustotheca* type. × 125.
Figs. 5, 6. Sporangia in basipetal series. × 80.
Figs. 7, 8. Five minutes after figure 7 was snapped the sporangium on the right burst, figure 8. Note difference in size before and after bursting. × 350.

PLATE 40

BREVILEGNIA SUBCLAVATA

Fig. 1. Sketch of sporangia and oogonia. × 134.
Fig. 2. Sporangia in basipetal series. All the sporangia have burst and discharged part of their spores. × 207.
Fig. 3. Habit of sporangia. × 134.
Fig. 4. Showing clustered habit of oogonia, and the remains of two sporangia. × 320.
Fig. 5. Habit of sporangia. × 134.
Fig. 6. Spores showing large central vacuole. × 854.
Fig. 7. Sporangia of a semiglobose shape. × 207.
Fig. 8. Oogonium showing three stages in egg formation: (a) Large vacuole before membrane broke; (b) disappearance stage; (c) egg rounding off. Five minute interval between drawings. × 573.
Figs. 9, 10, 11, 12. Oogonia, all but one with antheridia. Figs. 9, 10, 11 × 573; fig. 12 × 854.

PLATE 41

BREVILEGNIA SUBCLAVATA

Fig. 1. A young sporangium with a large central vacuole which extends down into the thread.
Fig. 2. The same thirty minutes later.
Fig. 3. The same thirty minutes later. Note change in the position of the vacuoles and the accumulation of protoplasm in the region where the basal wall is to be formed. The protoplasm in this region is of a greenish hyaline color.
Fig. 4. Five minutes later. Basal wall forming.
Fig. 5. Five minutes later. Basal wall formed. The vacuole elongating and beginning to extend out laterally.
Fig. 6. Forty minutes later. The vacuole pushing out still farther toward the wall.
Fig. 7. Ten minutes later. The spore initials formed with only a thin membrane lining the sporangial wall.

Fig. 8. Five minutes later. The membrane has broken and the spore initials are fusing.

Fig. 9. Ten minutes later. Spore initials completely disappeared. Note the numerous small vacuoles.

Fig. 10. Five to ten minutes later. Final spores appearing.

Fig. 11. Six minutes later. Spores swelling and almost completely disappearing again.

Fig. 12. Fifteen minutes later. Spores had swollen until sporangial wall had burst and several of the spores were shot out.

Fig. 13. Sporangium in about the same stage as shown in figure 6, killed and stained to show nuclei. Note there is no shrinkage. No very distinct nuclear membrane.

Fig. 14. Sporangium in about the stage shown in figure 10. Killed and stained as above. Considerable shrinkage. Spores uninucleate. The chromatin material apparently all massed in the central, deeply staining lump.

Figs. 1–12 × 640; figs. 13 and 14 × 1100

PLATE 42

BREVILEGNIA SUBCLAVATA

Fig. 1. Sporangia showing three in basipetal series. The two lower ones in the figure have formed spores part of which have been discharged. The upper one shows the spore origins at the time when the clefts have almost extended out to the sporangium wall.

Fig. 2. The same sporangium five minutes later. The clefts have extended out to the wall and the spore origins have passed into the disappearance stage.

Fig. 3. The same sporangium five minutes later showing the basal wall pushing inwards.

Fig. 4. An hour and a half later showing three clefts cutting the sporangial contents into four parts. Many small vacuoles present.

Fig. 5. Forty minutes later. One of the clefts has been completed, forming two large compound spores. The other two clefts failed to develop but are still visible.

Fig. 6. Thirty-five minutes later. The vacuoles have grown considerably and several have fused. The two compound spores are swelling causing a very noticeable distention in the sporangial wall.

Fig. 7. A few minutes later. The sporangial wall has burst on the side and one of the spores has popped out.

GEOLEGNIA SEPTISPORANGIA

Fig. 8. Young sporangium showing central vacuole and at its tip the thickened basal wall of an old sporangium formed in the same thread. Figure made 11:10.

Fig. 9. The same 11:50. Central vacuole now nearly obliterated, dense with protoplasm. Granules passing along lines. Three minutes after the figure was drawn the large mass of protoplasm by the arrow had passed into the sporangium.

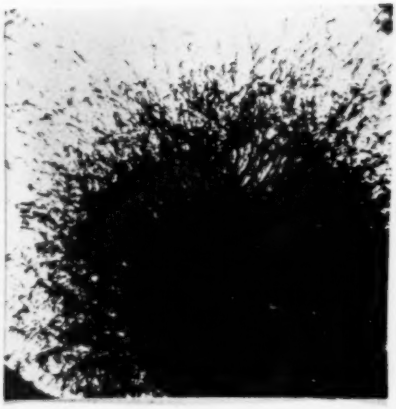
Figs. 10, 11. Twenty-four minutes later. Protoplasm by arrow moved up and formed wall, which was laid down three minutes after figure 10 was made (fig. 11). Wall straight. Note vacuole just above wall.
Fig. 12. One hour later. Many small vacuoles near center. Basal wall concave.
Fig. 13. Twenty-five minutes later. Basal wall convex. A large dense central mass being constricted into two parts. Note the apparently oval shaped vacuole surrounding the central mass.
Fig. 14. Fifteen minutes later. Central mass cut into two parts.
Fig. 15. Twelve minutes later. Peripheral layer of protoplasm becoming very thin. Two spore origins very distinct.
Fig. 16. Three minutes later. Spore initials disappeared.
Fig. 17. Three minutes later. Final spores beginning to appear, the spores rounding off and shrinking from the wall.
Fig. 18. Twelve minutes later. Final spores formed. Note the thickened cap like structure on the distal end of the sporangium, and the thickened wall at the base. Note also the swelling in the hypha beneath the old sporangium.
Fig. 19. Nuclei becoming massed in the center of what is to become a spore initial.
Fig. 20. Sporangium with three spore initials.
Fig. 21. Spores appearing as individuals just after the disappearance stage.
Fig. 22. Spores completely formed, showing nuclei still clustered in the center.
Fig. 23. Ripe spores, showing nuclei dispersed through the spore.

Figs. 1–7 × 320; figs. 8–18 × 640; figs. 19–23 × 1100

PLATE 43

GEOLEGNIA INFLATA

Fig. 1. Sporangium showing "spore initials." × 645.
Fig. 2. The same sporangium a few seconds after the lining membrane broke, showing the "spore initials" swelling. × 645.
Figs. 3, 4 and 5. Showing changes in position of spore initial by "a," and thinning down of connecting membrane. × 645.
Fig. 6. Connecting membrane now a delicate thread. Note vacuoles in spores. × 645.
Fig. 7–12. Development of a sporangium containing a single spore. × 360.
Fig. 13. Mature multinucleate spore. × 1238.
Fig. 14. Young sporangium showing multinucleate condition. × 1238.



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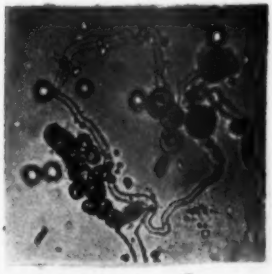

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BREVILEGNIA BISPORA

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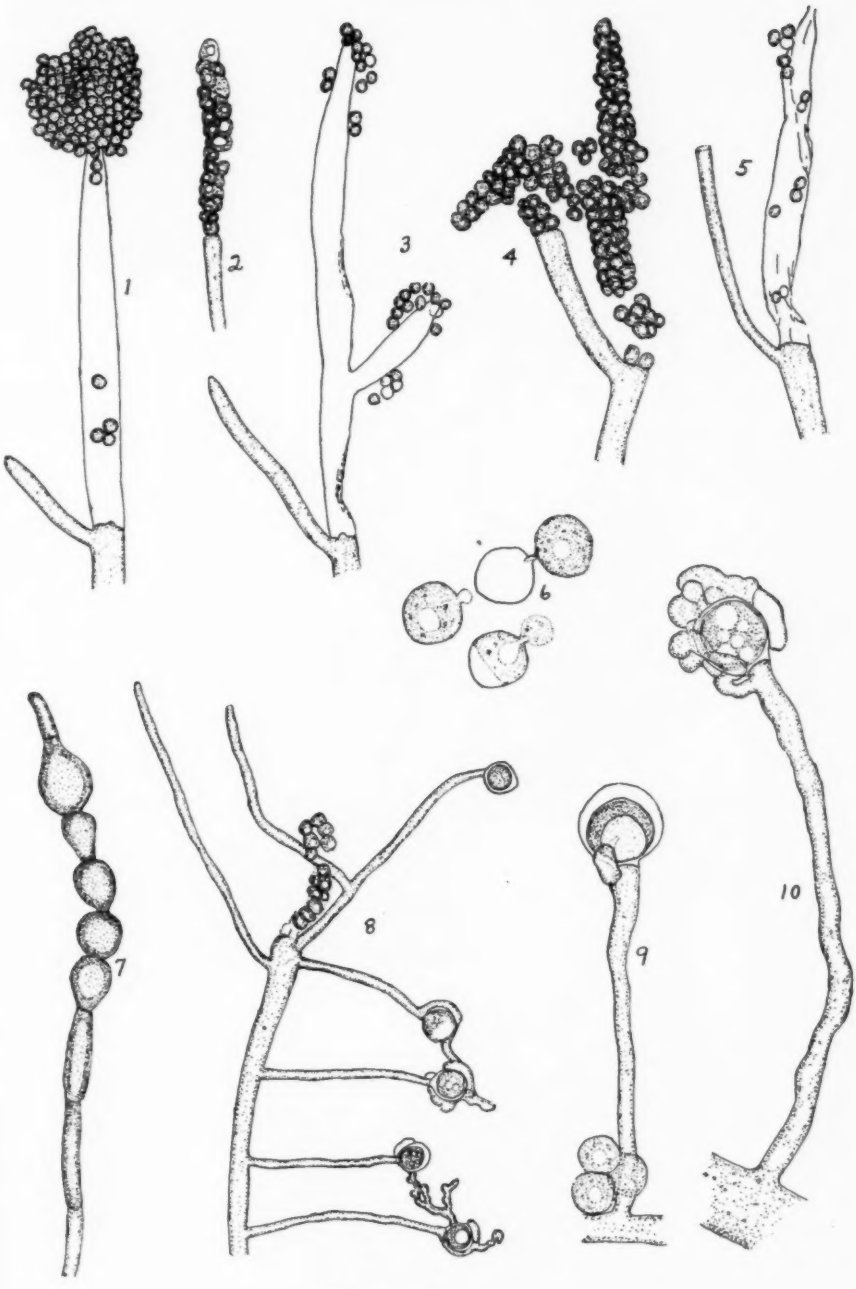
PLATE 38



BREVILEGNIA BISPORA

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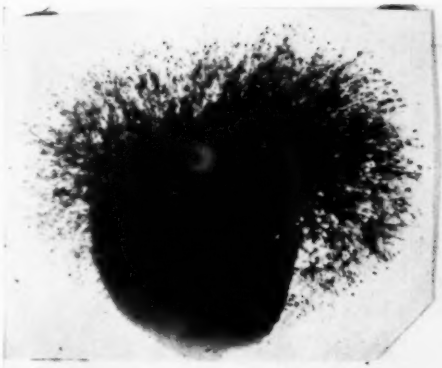

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BREVILEGNIA SUBCLAVATA

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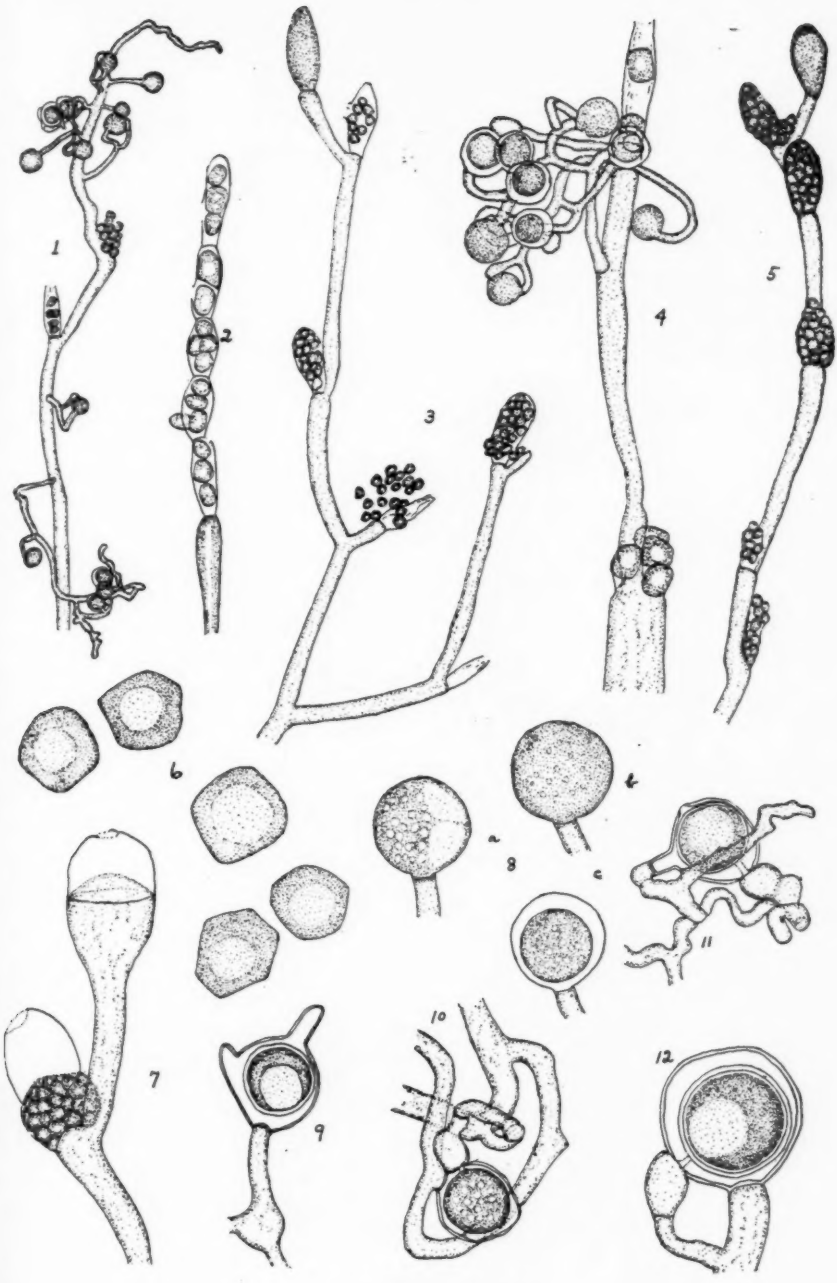
PLATE 40



BREVILEGNIA SUBCLAVATA

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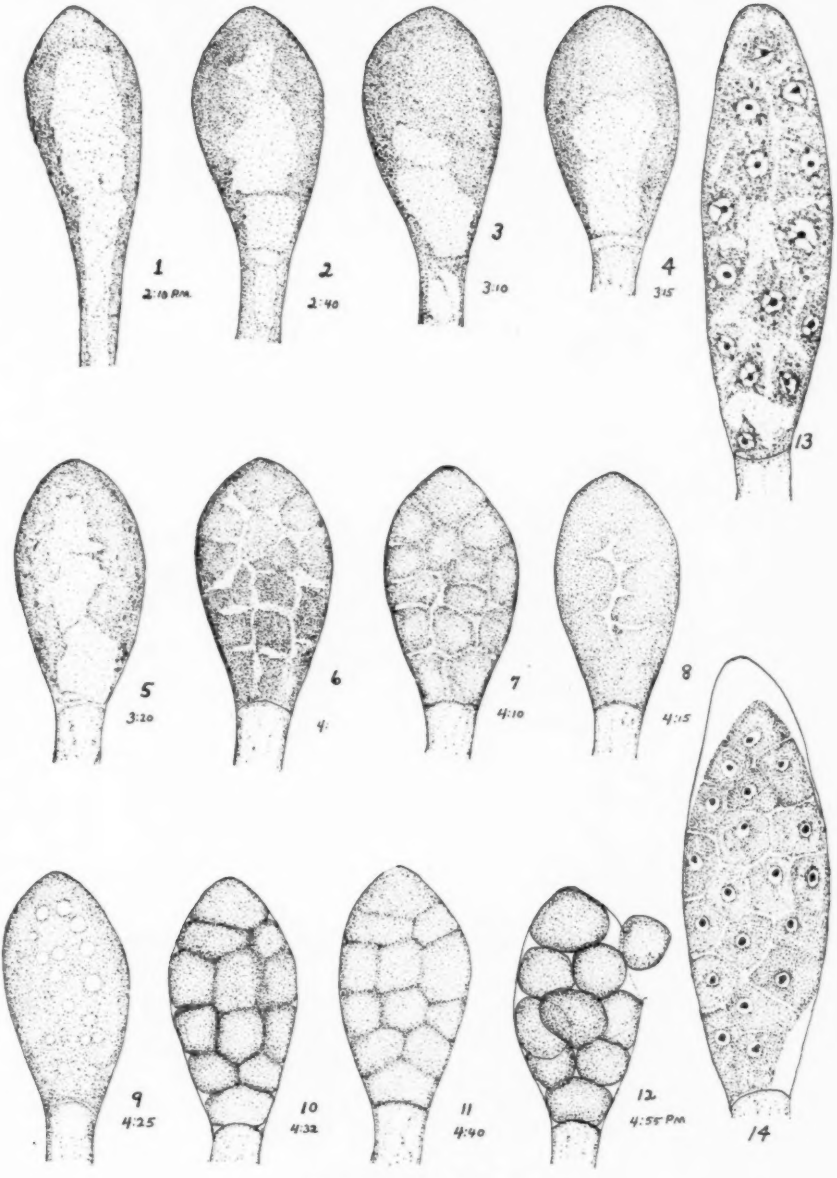
PLATE 41



BREVILEGNIA SUBCLAVATA

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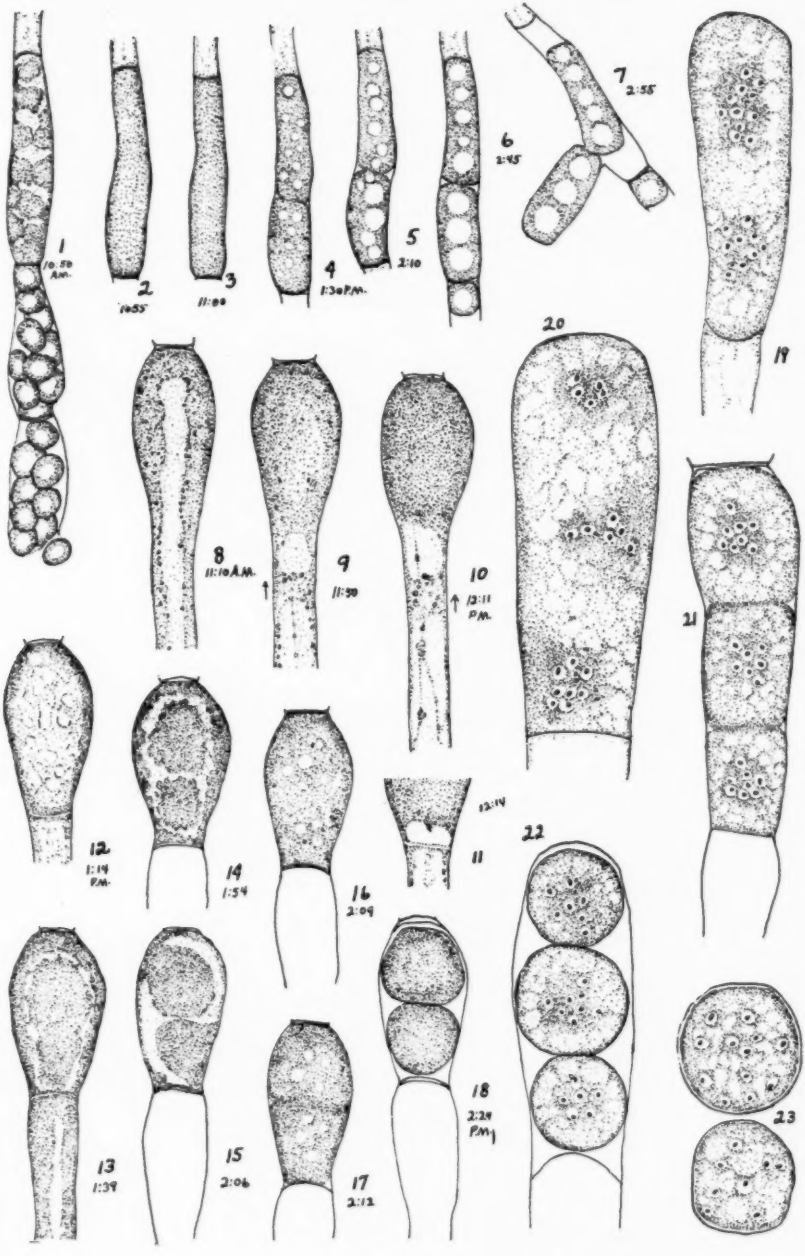
PLATE 42



BREVILEGNIA SUBCLAVATA, FIGS. 1–7
GEOLEGNIA SEPTISPORANGIA, FIGS. 8–23

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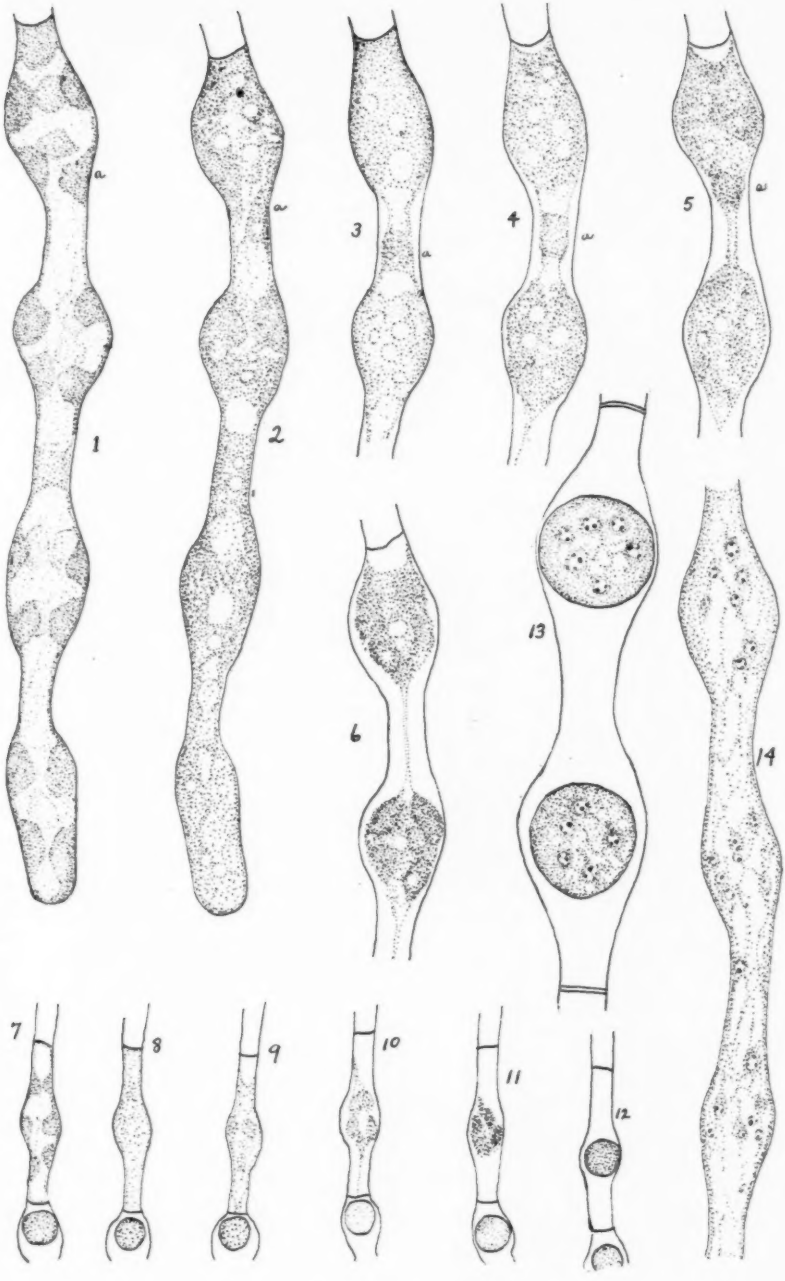
PLATE 43



GEOLEGNIA INFLATA

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BREVILEGNIA DICLINA N. SP.

By James Vernon Harvey

Plates 44 and 45

Mycelium dense and rather opaque, of fast but limited growth and having much the appearance of *Geolegnia* species. On boiled hemp seed in water reaching a diameter of 1 cm. in about five days and 15 mm. in two weeks. Hyphae straight, sparingly branched, at first resembling *Leptolegnia*, 5–28μ thick, usually 10–13μ. Primary sporangia formed within one day, singly or in clusters at the tips of practically all hyphae, sometimes many arising from the same point in dense sympodial groups; ovate to long club-shaped as in *Thraustotheca clavata* or sometimes long and slender with the spores in a single row throughout or in part. Secondary sporangia dense in sympodial clusters and also not rarely from segments cut off below the primary ones. Spores spherical or oval to elongated, frequently angular from crowding, most of the smaller ones about 10.3μ thick or if elongated 10.3–12.8 $\times$ 12.8–25.6μ, larger ones with a central clear spot as in *Geolegnia inflata;* escaping by dissolution of the sporangium wall, then sprouting with a germ tube, a swimming stage lacking. [Prof. Couch has recently found that the spores can be made to swim by putting them in distilled water adjusted to a pH about 4, or filtered through animal charcoal (pl. 44, fig. 3). W. C. C.] Oogonia abundant 21–33μ, mostly 21–25μ thick, borne singly and apically on branches smaller than the main hyphae, often as in *Achlya caroliniana;* formed usually after one week, often within three days; spherical to irregular and often with few to many projections which may reach 36μ in length, rarely over 5.5μ in breadth, wall about 1μ thick; earlier oogonia often proliferating, the contents forming a new oogonium at the tip, which may mature an egg. Eggs one to each oogonium, 18–25μ, mostly 18–21μ thick, spherical, usually filling to oogonium, though often not filling it, eccentric with a large lateral oil drop which is entirely surrounded by protoplasm; egg wall up to 2μ thick. Mature eggs never found in oogonia bearing projections, and mostly in the spherical ones. Antheridia more often absent, when present only one and usually diclinous as in *T. clavata* and closely resembling that form in that the antheridial stalks are long, irregular and branched; rarely androgynous, in which case the antheridial stalks may be much coiled and irregularly wrapped about the oogonium. Fertilization apparently does not take place.

Found eight times in 200 collections between June 23 and July 23, 1925; in dry soil (humus and loam) three times and in moist soil (humus, black-sandy, and bick-filled) five times; depths: surface, 2 in., 6 in., and 9 in.; under such vegetation as water-leaf, crowfoot, Virginia creeper, dandelion, grasses, ferns, and mosses; open fields and woods; widely scattered areas around Madison, Wis.

The methods of collection and culture are as described in a recent paper by the author (2). In the original cultures the plant was usually found alone on a bit of substratum, although other pieces of the same substratum in the same culture at times bore *Pythium* species, *Geolegnia* species or certain Ascomycetes or Zygomycetes. The Ascomycete forms easily infest our new plant, but other forms do not. In fact, when first isolated, one culture, containing five pieces of hemp inoculated with the new species became strongly infested with *Pythium proliferum*. This culture was set aside to be used as a *Pythium* culture, which when observed after 20 days showed that the strong *Pythium* growth had been overcome by the hardy growth of the new plant, which bore oogonia, some of the oogonia having ripe eggs; there were also many sporangia. Ring growth had not reached over 10 mm. Bacterial infections are easily survived.

Upon first observation the plant resembles *Thraustotheca clavata* (I), but further observation shows the mycelium to be more densely matted, like that of *Geolegnia*; however, the mat being more transparent than in those species. The matted portion owes its existence to interlacing hyphae, hyphae bearing oogonia, and a few sporangia, as found to be the case in *Geolegnia*, plus the smaller, more delicate, rapidly growing hyphae of the abundant sprouting spores. The spores sprout easily in position when fresh water is placed in the cultures or when spores are placed on corn meal agar. In water the spores often sprout with long slender, unbranched germ tubes, which interlace with the parent hyphae, causing the plant to appear infected with some other plant, such as a *Pythium*, and adding to the density of the mycelium and obscuring the parent threads except at the periphery. Spores sprout easily on agar, but the germ tube does not grow to such a great extent as in water cultures, often ending in an irregularly knotted body, resembling an abortive oogonium as seen in many cultures.

On corn meal agar the plant may reach a ring growth of 5 cm. within two weeks, in this case very few sporangia and no oogonia being formed. The plant then to the naked eye resembles more closely a delicate *Achlya*, the threads radiating in all directions, fairly straight, long and

slender and very sparingly branched, usually about 8–12μ thick. This is the result when an agar plate is inoculated with a block of agar containing mycelium or sprouting spores of the plant. If a bit of hemp seed with a growth of the plant is placed on the agar the growth on the agar will be small, the ring growth not reaching over 18 mm. in ten days, and in the cases observed, not growing to any greater extent by the end of four weeks. Very many sporangia in large clusters, up to 25 in number, are formed, all arising near the same point, or sporangia may be formed end to end on a single hypha, up to 5 in number, the sporangia in each of the above cases being about the usual size, 10–30 x 60–100μ. They are long barrel-shaped, short or long club-shaped or rarely approach filiform. Intercalary sporangia are seldom found on agar, though often in water.

The plant grows well on bits of seeds of radish, sunflower, and buckwheat, as well as bits of grains of rye and wheat. All of these substrata produce clean and clear-cut growths of the plant, some even better than hemp. The most healthful growth is on buckwheat where sporangia and oogonia are produced in abundance. Radish, sunflower seeds, rye and wheat producing growths about like hemp. Corn offers a poor substratum. On buckwheat the plant to a great extent resembles *Thraustotheca clavata* in habit, with many small, branching, gnarled threads, with oogonia borne much as in *Achlya caroliniana;* here the sporangia are more like the true *Thraustotheca* type. Oogonia on all substrata produced within five days.

In the sporangia with more than one row of spores maturation takes place as in *Thraustotheca* (3) or all the common water molds with large sporangia, by the central vacuole cutting out blocks of protoplasm which round up to produce spores. In the case of a single row of spores, maturation takes place as in *Geolegnia septisporangia* (2) by the ingrowth of vacuolate furrows, cutting out blocks of protoplasm in a single row which usually round up to produce spores, though if crowded sometimes remaining more cubical or rectangular.

The first oogonia to be noticed were found in a discarded culture, which had been set aside for about three weeks. They contained no eggs and had many irregular protrusions, dense with protoplasm. The oogonia, which may proliferate through one of these projections, may produce a second, or even a third oogonium by proliferation from the second, the proliferating ceasing when a mature egg is formed within the last. Eggs are perfectly spherical and rarely ever found in any oogonium that has projections or is not spherical and if found in oogonia

bearing projections never extending into the projections as in *L. subterranea*. The lateral oil drop results from the accumulation of many smaller droplets scattered throughout the protoplasm of the egg. Antheridia seldom observed in contact with young oogonia; found on about half of the oogonia in older cultures, and in half of these cases always arising from a hypha remote from the hypha bearing the oogonium; sometimes androgynous. When no antheridia are found the oogonial habit closely resembles that of *A. caroliniana*, with many short stalks, some curving around the main hypha, bearing at their tip an oogonium.

SHAWNEE, OKLA.

EXPLANATION OF PLATES

PLATE 44

BREVILEGNIA DICLINA

Figs. 3 and 9 by J. N. Couch

Fig. 1. A portion of a hypha showing an intercalary sporangium and habit of oogonia. × 330.

Fig. 2 and 4–8. Types of sporangia. × 310.

Fig. 3. (a) Sporangium where a few spores have emerged from cysts. PH_4 solution. × 420.

(b) Spore emerging from cyst. × 420.

(c) Spore emerged from cyst. × 420.

(d) Emerged spore with cilia. × 420.

(e) Swimming stage of spore. Killed with iodine solution. Shape unlike live spore. × 825.

Fig. 9. Sporangium. Spores as in *Geolegnia septisporangia*. × 155.

Fig. 10. A maturing sporangium of the clavate type. × 200.

Fig. 11. A maturing sporangium of the elongated type, in which spores are formed in a single row. × 200.

Fig. 12. Sketch to show branching habit, a typical method in which sporangia are renewed. × 200.

Fig. 13. Spores sprouting in position. Also to show habit of oogonia. × 310.

PLATE 45

BREVILEGNIA DICLINA

Fig. 1. Showing proliferation of old oogonium to form a new one. × 555.

Fig. 2. Oogonium with protrusions. Also to show anteridium. × 625.

Figs. 3 and 4. To show habit of oogonia (immature). × 372.

Fig. 5. Hypha with typically knotted branch. × 372.

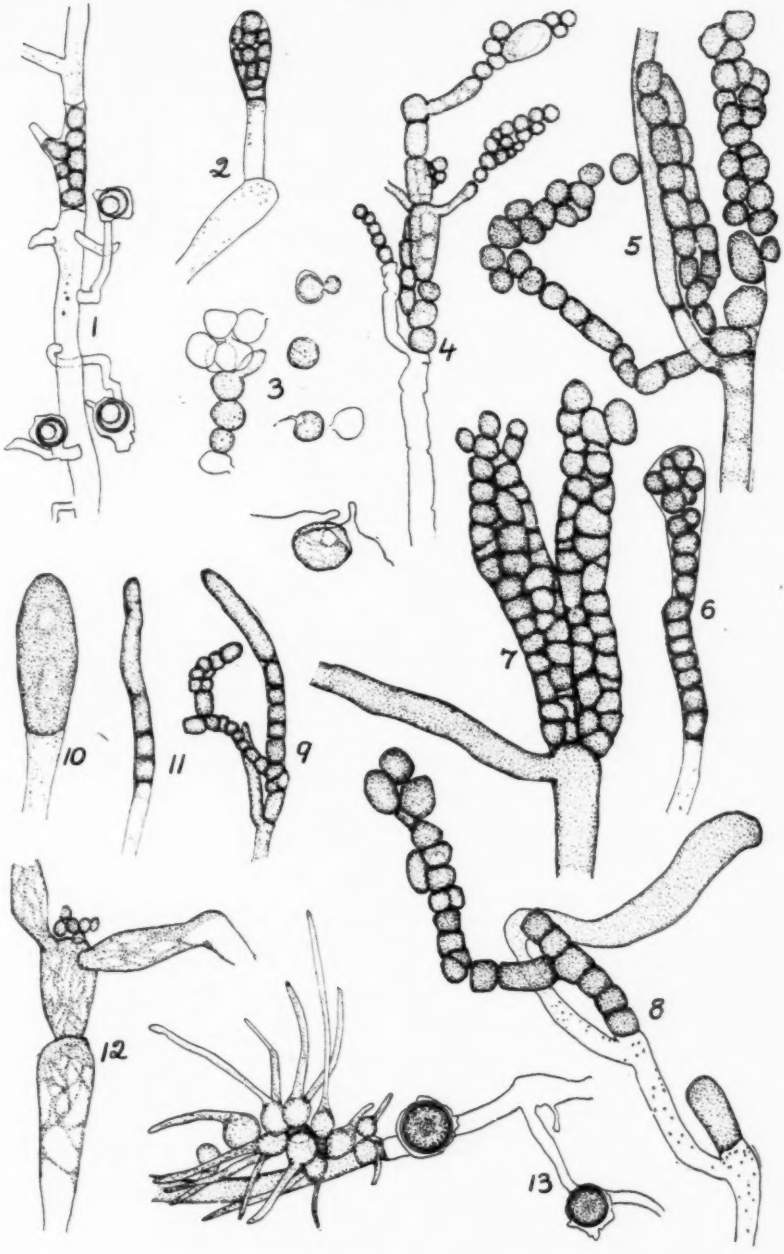
Figs. 6–8. Various stages in egg formation. 6, 7 × 625; 8 × 752.

Figs. 9–11. Oogonia with ripe eggs and diclinous antheridia. × 684.

Fig. 12. Proliferated oogonium. × 372.

Fig. 13. Habit of plant. × About 100.

PLATE 44



BREVILEGNIA DICLINA

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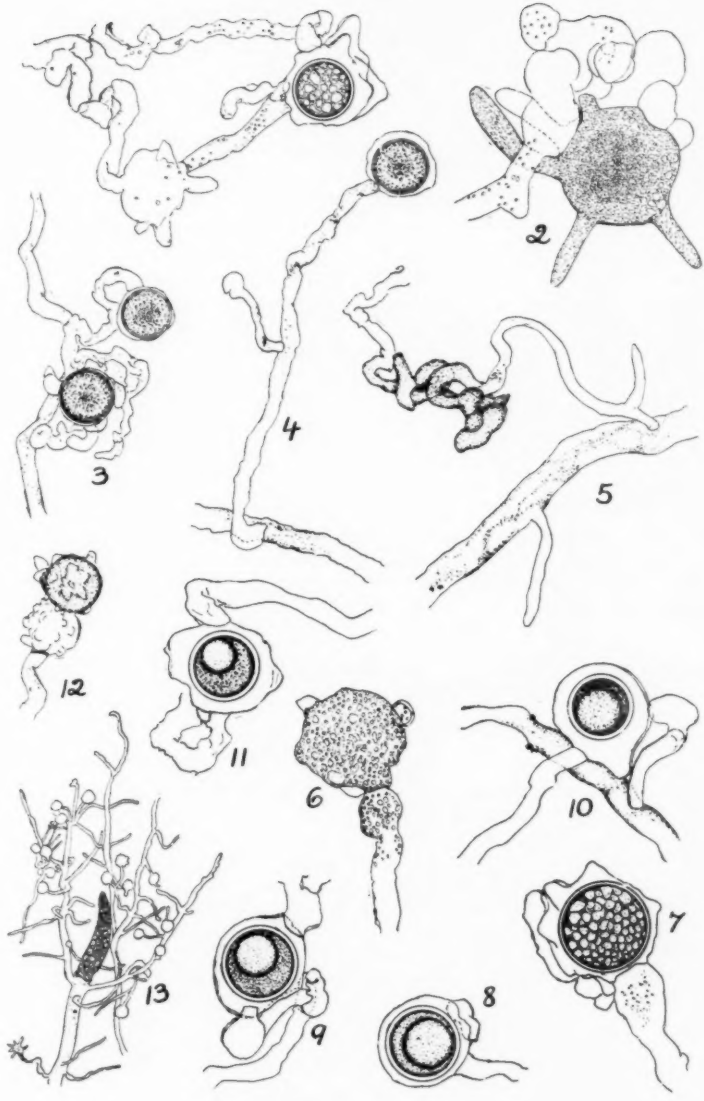
PLATE 45



BREVILEGNIA DICLINA

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A NEW SPECIES OF PYTHIUM

By W. C. Coker and P. M. Patterson

Pythium torulosum n.sp.

Plate 46

Mycelium dense, submerged in water cultures. Hyphae irregularly branched, 2.5–4μ in thickness. Sporangia consisting of few to many large, tuberous and branched swellings much thicker than the hyphae, and when fully formed, filled with dense protoplasm and cut off by septa from the rest of the plant. The contents of these growths empty through a filamentous portion of the sporangium to a typically *Pythium* vesicle where the spores are formed. Zoospores 15–20 in number, 7–8μ in diameter when encysted. Oogonia terminal, intercalary or stalked; smooth, spherical, 12–19μ, mostly 17–18μ, wall very thin. Eggs entirely filling the oogonia, their hyaline walls rather thick (0.8μ), the contents when ripe having an emulsified appearance with most of the oil contents near the center; antheridial branches short, androgynous, arising from the oogonial stalk, or less often from the same hypha nearby, rarely branched; antheridia tuberous, occasionally more than one to the oogonium.

According to Butler's arrangement of the genus *Pythium*, our species belongs in the subgenus *Aphragmium*. It is closest to *P. gracile* Schenk. and *P. monospermum* Pringsh. *Pythium torulosum* differs from *P. gracile* in the following ways:

1. Larger, more swollen and more conspicuous sporangial complexes. This also distinguishes it from all other species.
2. Smaller oogonia, 12–19μ (*P. gracile*, 15–33μ).
3. Antheridia closely androgynous (*P. gracile* usually diclinous).
4. Eggs completely filling the oogonium (do not in *P. gracile*). Eggs about the same size, but with the upper limit about 6μ less in our plant.
5. Hyphae much larger than in the saprophytic form (b) of Butler, and only equalled in size by form (c), parasitic on the roots of ginger and castor bean. This last form is now known as *P. Butleri*.

From *P. monospermum* the present species differs in the much smaller threads of the vegetative mycelium (half the size) and in the proportionately much larger and more swollen sporangial elements. The

lateral processes of *P. monospermum* are more cylindrical and not noticeably larger than the regular hyphae. In both cases, complex and extensive branches of hyphae are formed. Again our plant differs from *P. monospermum* in that the antheridia in the latter are diclinous for the most part (that is, as far as is shown in the drawings). The oogonia of *P. monospermum* are about the same size as in ours, though in our form the egg completely fills the oogonium.

Pythium torulosum appeared about March 5, 1926, in a series of cultures made from mosses and liverworts in search of water molds. It was found the same day in two cultures, one (type) from *Teleranea nematodes* (Gottsche) Howe and *Thuidium delicatulum* (L) Mitt, both collected (Chapel Hill, N. C.) three months previously and kept to the time of culturing in a state of desiccation on the laboratory table. It was found again in a water culture on April 10, 1926, at Goose Creek about 17 miles west of Charleston, S. C. (Coker, coll.; isolated by Miss Matthews), and again in a water culture from small stream 4½ miles south of Wilmington, N. C., on Jan. 28, 1927 (H. T. Thompson, coll.). The specific name chosen refers to the torulose or conglomerate sporangial masses.

In order to get a pure culture a single spore of the plant was placed on a sterilized agar plate. When the mycelium had spread over a convenient area, small blocks of agar containing some of the mycelial growth were transferred. If boiled pieces of corn or hemp seeds in water are inoculated from a single spore culture on agar, in two or three days the swellings of the sporangial initials give the mycelium a characteristic knotty appearance. These protoplasmic containers are formed directly off of a portion of a hypha that is to become a sporangium, or are formed by a swelling in the hypha, or by short branched hyphae that in turn become greatly distended and knotted as the protoplasm is packed into them. These unit masses of connected containers may be either terminal or intercalary and when fully grown are cut off by one or two walls (depending on their position) from the original hypha that bore them. Sometimes these tuberous outgrowths form very complicated masses and are impossible to trace out; only the unusually simple sporangial growths have been figured. There is a filamentous portion formed (in one case measuring 300μ in length) through which all the contents of a group of containers pass into an apical vesicle.

The following notes concern the subsequent events in a typical case: There was a slight contraction of the protoplasm away from the wall of the vesicle after discharge. The protoplasm was becoming more

coarsely granular and after 3½ minutes a slight movement of the whole body of the protoplasm was detected. After another ½ minute, this unified movement gave way to a vague movement of several undifferentiated parts of the whole mass. After another 1½ minutes, fissures outlining the spore appeared. After a further 3 minutes, individual spore movements commenced, though as yet all the spores were attached to each other by strands of protoplasm. One and one-half minutes later, the spores were completely rounded and were swarming among each other. The motion of the spores at first slow, gradually accelerated in speed for the next 4 minutes, until the wall of the vesicle bulged at the impact of each spore. About 10 seconds before discharge of the spores, the movements suddenly became violent, rupturing the wall of the vesicle in an irregular tear, and all the spores left the vesicle in about a second; this occurred 15½ minutes after the discharge of the protoplasm from the sporangial containers. Time 8:30 P.M., temperature of culture 25°C., substratum, piece of corn grain.

Pythium torulosum grown on peptone agar formed a sparse growth, and when the culture was old had packed all of its protoplasm into the characteristic sporangia. It then went into a resting condition without the discharge of any spores or formation of any oogonia. When transferred to distilled water, vesicles were formed and spores discharged in 3–4 hours. Within the agar the spores are discharged but cannot swim, and many of the spores are broken at the time of discharge within the agar. On maltose-peptone agar, a dense growth occurred, and a great number of oogonia but no sporangia were formed. On corn meal agar, sporangia were first formed and the oogonia later, the latter often arising from resting sporangia.

This species has been cultivated on various media for over a year. During all this time the species has had the same characteristic growth and appearance and the measurements for the different parts are the same now as when first made.

The following innoculation tests indicated the inability of *P. torulosa* to "damp off" seedlings. Sterilized seeds of peanut, Pinta bean, black-eye pea, cabbage, and lettuce were sprouted in sterilized soil, and watered with autoclaved water. All of these cultures were kept in a closed case that had been previously washed out with a solution of mercuric cloride. When the seeds had sprouted, mycelia of *P. torulosa* (grown on hemp seed in water) that had gone into resting condition, were transferred with flamed utensils to the seedlings, about ¼ of an inch above the soil. These cultures were covered with a bell jar containing air

saturated with water vapor. Parallel, uninoculated cultures were kept for controls. No "damping off" occurred in any of the cultures. The fact that the fungus was in a resting condition may have been a possible factor in preventing "damping off" in the first series of cultures, so the experiment was repeated under the same conditions and with the same precautions, with actively growing mycelium of *P. torulosa*, using only the seedlings of cabbage and lettuce. *Pythium torulosa* was placed on a maltose-peptone agar medium and grown entirely free from bacteria. By the time it had spread an inch from the point of cultural transference, thus being in a very actively growing state, it was transferred in small blocks of agar ($\frac{1}{8}$ x $\frac{1}{8}$ x $\frac{1}{4}$ in.) to the seedlings, on or near the ground and kept damp under a bell jar. The mycelium grew about the hypocotyl and into the soil, but did not attack the seedlings.

CHAPEL HILL, N. C.

EXPLANATION OF PLATE 46

PYTHIUM TORULOSUM

Fig. 1. Habit of mycelium with partially developed sporangial swellings from which probably two or three sporangia will be formed.

Fig. 2. Fully developed sporangial complex, representing a single sporangium. Two filaments are being given off, one of which will discharge the spores.

Fig. 3. A fully developed sporangial group representing two or probably three sporangia.

Fig. 4. A single sporangial group after discharge, the tube of discharge is cut off at the right. It was three or four times as long as the part shown.

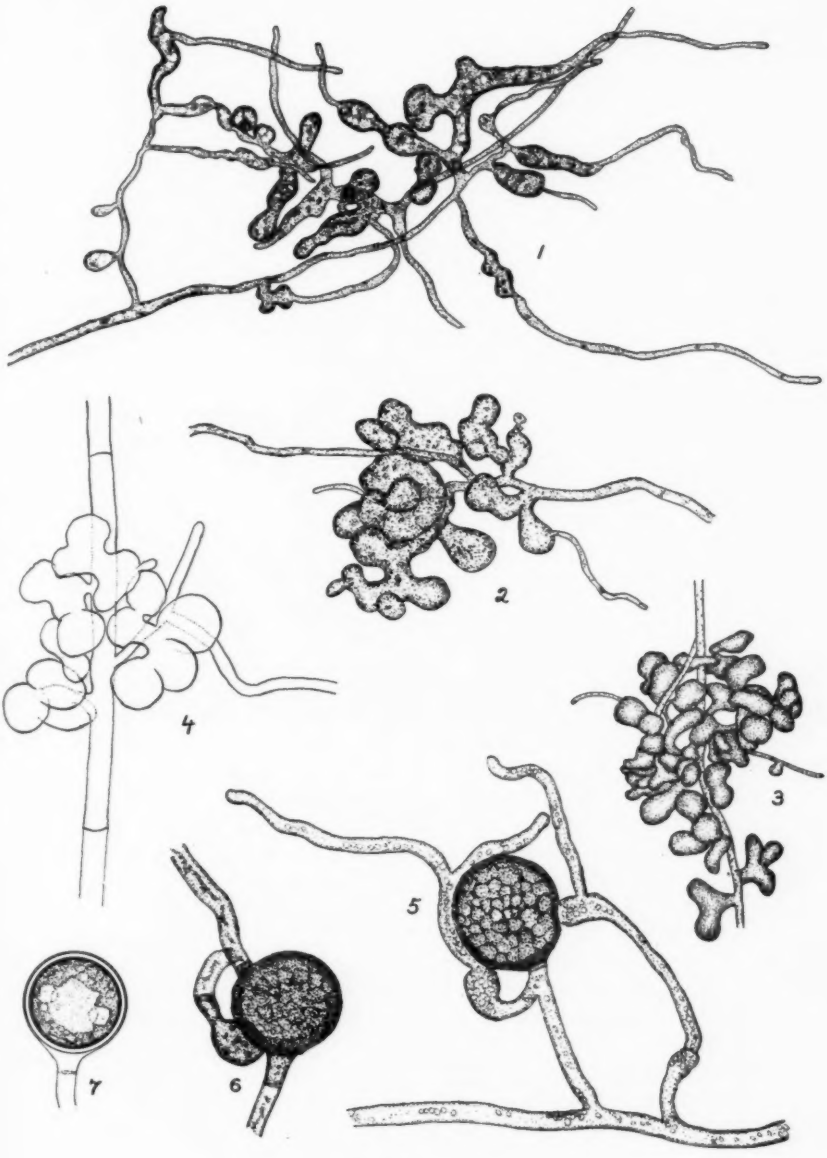
Fig. 5. Young oogonium with two antheridia that have sent off branches. Unusual.

Fig. 6. Young oogonium with a single antheridium of normal appearance.

Fig. 7. Oogonium containing a ripe egg which completely fills it.

Figs. 1 and 2 × 577; fig. 3 × 367; fig. 4 × 540; figs. 5–7 × 667

PLATE 46



PYTHIUM TORULOSUM.

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NEW OR NOTEWORTHY BASIDIOMYCETES

By W. C. Coker

Plates 47–49

Clavaria Stillingeri n.sp.

Plate 47

Plants small, single, arising in an open colony from a dense, continuous or interrupted mat of white, cottony mycelium (subiculum), without obvious rhizomorphs; simple or once or twice openly branched or forked above, terete, 0.8–2 cm. high and up to 1.5 mm. thick, the branches spreading, antler-like and tapering to sharp points; color when dry dull brown (about wood brown), the tips whitish when young; surface smooth. Flesh pale, rigid and moderately brittle when dry; tasteless.

Spores ochraceous under the microscope, smooth, elliptic (not bent), and with an eccentric mucro, 4.4–5.5 × 8.5–12.5μ. Basidia club-shaped, 7.5–9.5 × 25–34μ, with 4 distinct sterigmata up to 7.4μ long. Hymenium entirely confined to the upright fruit bodies, the basal mat bearing no spores.

Among the species known to me this is nearest *C. byssiseda* and *C. epichnoa*. From the former it differs in smaller size, much simpler form (few or no branches), and quite different spores; from the latter in the stouter body, larger spores and more obvious mycelial mat. *Clavaria myceliosa*, a small western plant growing on coniferous leaves, has a more delicate structure, very small papillate spores, and belongs to a different group. *Clavaria pinicola* Burt has very similar spores but otherwise is not closely related, being a much larger, more complicated plant of different appearance and mycelium composed of obvious rhizomorphs.

Clavaria byssacea is a rare European plant that from the figures and description might be thought to approach the present species. It seems to have been figured only in the original publication (Roth in Usteri; Annalen der Bot., pl. 1, fig. 1. 1791). The two drawings, one enlarged, look more like the quite simple *Hydnum Himantia* Schw. (*H. Cookei* Berk.), except that the plants are drawn as standing up instead of hanging down. The plant treated as *C. byssacea* by Bourdot

and Galzin (Bull. Soc. Myc. Fr. **26**: 216. 1910) is *H. Himantia* as shown by a good collection sent me by Abbé Bourdot and so recognized by him on the label. It is obvious that while our plant is a very small *Clavaria* it is still much larger and distinctly more branched than *H. Himantia* (*C. byssacea*). In size and appearance it is intermediate between the resupinate Hydnums (as *H. Himantia*) on the one hand and such Clavarias as *C. byssiseda* on the other. Small and simple specimens of our *Clavaria* could be distinguished from a *Hydnum* only by the absence of a hymenium on the subiculum between the teeth and by the upright habit. As *Mucronella* has no hymenium between (and practically no subiculum in most forms) the pendent habit is about all that is left to distinguish the resupinate Hydnums from *Clavaria*.

Idaho. Elk River. On wood of *Abies grandis*. C. R. Stillinger, coll. (U. N. C. Herb. from Lloyd Herb., type; Lloyd Herb., cotype.).

Lachnocladium pusillum n.sp.

Plate 49

Growing on ground; very small, cespitose or crowded in small clumps up to 5 mm. wide and 7 mm. high, stalked and branching simultaneously into several more or less spreading terminal prongs which are bluntly pointed; terete except toward the flattened angles, smooth (glabrous) throughout; hymenium borne on all sides (amphigenous). Color reddish-cartilaginous, subtranslucent above. Texture tough, pliable and cartilaginous.

Spores smooth, spherical, 4.8–6μ thick, with a large oil drop and a small mucro. Basidia clavate, 5.5–7.5μ thick with 2–4 curved sterigmata about 4.5μ long. Threads of flesh 1–1.5μ thick, densely packed, parallel.

Holland. Leiden. In a pot containing *Rhamnus* in a hothouse. Mr. Lagendijk, coll. (Sent by Miss Catherine Cool, Sept. 1925) (U. N. C. Herb.; type). Also another coll. (Oct. 12, 1926) from the same place.

This little species is of great interest as it is the second *Lachnocladium* to be recognized in Europe (see Coker, The Clavarias of U. S. and Canada, p. 198). It was sent to us by Miss Catherine Cool together with several other fungi collected in the neighborhood of Leiden. The description is made entirely from the dried condition, as no notes accompany the plants.

As observed in our Clavarias of the United States and Canada, p. 198, there is in the Bresadola Herbarium a plant from Sweden, determined by him as *Merisma tuberosa* Grev., which is the same as *Clavaria gigaspora* Cotton, and really a *Lachnocladium* closely related to *L. semivesti-*

tum. This plant from England and Sweden is the only *Lachnocladium* hitherto recognized in Europe. It is easily distinguished from the present plant by much larger size and different spores. It is interesting to note the similarity of the basidia of this plant to those of the American and English species.

The genus *Lachnocladium* at present contains a number of very distinct groups only remotely related to each other, and is in great need of revision. Patouillard in his Essai taxonomique divided the genus into two sections, *Dendrocladium*, with rough or asperulate spores, and *Coniocladium*, with smooth spores. The present species would of course fall into the *Coniocladium* group. This group, however, is not a very natural one as it contains such widely different plants as *L. Braziliense* and *L. cartilagineum*. The sub-group into which *L. pusillum* falls contains *L. semivestitum*, *L. cartilagineum* and *L. gigaspora*. The group is characterized by the dense, homogeneous, cartilaginous flesh, smooth (glabrous) surface (the tips may be pilose in growth), smooth, pure white spores, basidia with 2–4 long, curved sterigmata, and by the terrestrial habit. *Lachnocladium vitellinum* Pat. from China is much like this group except for the brighter color and drab spores (Revue Myc., 1890, p. 134, pl. 107, fig. 3). It is the only *Lachnocladium* smaller than the present species.

Lachnocladium Micheneri B. & C.

Plates 47 and 49

The following description is made entirely from dried plants.

Emerging from mixed oak and hemlock leaves and trash; branched from the ground and repeatedly dividing to form very dense clusters up to 3 cm. high and 2.8 cm. thick, connected below with obvious white mycelium; bases scurfy, but the branches with hymenium glabrous. Tips very acute and often flattened. Color about dull wood brown, tips concolorous or somewhat paler. Flesh pliable and somewhat toughish but not at all cartilaginous, concolorous; odorless and tasteless.

Spores apparently white, smooth, subelliptic, 3.6–4 × 6–8.5μ. Basidia 4-spored, 7.6μ thick.

Lachnocladium Micheneri is a much smaller and more delicate plant than *C. stricta* and the spores are, under the microscope, hyaline, thin-walled, and quite smooth, differing in appearance from those of *C.*

stricta when seen side by side. The plant is about the size of *C. byssiseda* but is distinctly darker, less stalked and as a rule more densely branched. The spores, of course, are quite different. Hard's figure 401 (Mushrooms, p. 476), as *L. Micheneri*, we take to be *C. byssiseda.*

This is the first collection we have seen that can be referred to *L. Micheneri.* The plants agree both in appearance and spores with those figured by Burt under that name (Ann. Mo. Bot. Garden **6**: 270, fig. 9, and pl. 5, fig. 3). Burt considers *C. fragrans* Ellis and Ev. the same, but we think the latter is *C. gracilis.*

Illustrations: Burt. As cited above.
Ohio. Burtt Leeper, coll. (Lloyd Herb. No. 11, and U. N. C. Herb.).

Geaster saccatus var. **Walkeri** n.var.

Plate 49

We have recently received from Miss Leva B. Walker of the University of Nebraska a set of Geasters, among which was a plant so peculiar as to merit some notice. The buttons are of the *saccatus* type, attached by a strictly basal mycelium and formed outside the substratum, therefore with a clean surface. On the other hand, there is no sign of any definite peristome. The plants are a very dark brown color on the outside, contrasting strongly with the paler spore sac. We will describe this as follows:

Buttons subspherical with or without a small blunt point, gregarious to subcaespitose, 1–1.5 cm. thick, basally attached to an obvious floccose mycelium, surface felt-like, not strigose, very dark brown (Verona brown of Ridgway), splitting into 6 or 7 bluntish rays which extend only about $\frac{1}{3}$ to $\frac{1}{2}$ the distance and confine the spore sac to a deep cup; outer layer not at all separable. Fleshy layer when dry varying from dark brown to much paler. Spore sac grayish brown, the mouth without a peristome, fimbriated about as in *G. fimbriatus.* Spores minute, faintly rough, 2–3.2μ. Collected near Lincoln, Nebr., fall of 1926 (Leva B. Walker, No. 4).

The plant differs from *G. fimbriatus* in entirely different habit (basal attachment of exposed buttons and inseparable spongy surface) and from *G. saccatus* and its relatives in absence of peristome and in very dark color. The plants are larger than any species of the *saccatus* group except *saccatus* itself. The spores are smaller than any of the *saccatus* group except what we have called the small spored northern form of *saccatus.*

Miss Walker writes that "all of the colors had a beautiful rosy tint to them in the fresh condition."

Boletus parasiticus Bull.

Plates 48 and 49

This peculiar *Boletus*, parasitic on *Scleroderma*, and the only parasite in the genus, has not before been reported south of Pennsylvania. During the summer of 1922 we found good specimens of it in the mountains of this state on mossy humus in mixed woods near a large hemlock tree. Our plate 48 is of these plants.

The cap was dull yellow in color with the texture of leather; tubes yellowish; and in our specimens the whitish flesh turned yellow when cut. The spores are smooth, elliptic, 4.2–5 × 11.5–15.5μ.

North Carolina. Blowing Rock. In mossy humus, summer of 1922. Coker, coll. (U. N. C. Herb.).

Reported heretofore in America from New England, New York and Pennsylvania.

ARACHNIACEAE Coker and Couch

Although the genus *Arachnion* was established by Schweinitz over a hundred years ago, its internal structure and the development of its peridioles has up to the present remained quite unknown. As a result its position in the Gasteromycetes has likewise been unknown. Fischer (Engler and Prantl, Pflanzenfamilien) puts the genus among several in a doubtful group at the end of the Sclerodermataceae, evidently because of its peridioles. We have recently studied its development and can now say with certainty that the genus is not closely related to the Sclerodermataceae, but is nearest the Lycoperdaceae. It cannot be placed in that family, however, and there is nothing left to do but give it a family of its own. This we define as follows:

Fruit bodies exposed: peridium single, very thin, crumbling at maturity. Gleba with distinct chambers lined by the hymenium, these chambers forming at maturity a mass of minute, separate, hollow peridioles which crumble and escape like grains of sand. Capillitium and sterile base none.

This family is most nearly related to the Lycoperdaceae, from which it differs in the single peridium, absence of capillitium, and presence of peridioles. The peridioles in *Pisolithus* are of a very different nature,

being solid masses at all stages and without hymenium. We are including in the family only the genus *Arachnion*, as *Arachniopsis* and *Holocotylon* are not yet well enough known to be placed. If the former genus is good and Long's description is accurate, it is very different from *Arachnion*, and if Lloyd is right in thinking it the same as *Arachnion* (Myc. Notes, p. 1134) it need not be considered. *Holocotylon* appears to be related to *Arachnion*, and if so the family description will have to be modified by the omission of peridioles. If the South African plant described by Lloyd as *A. Scleroderma* is really related it will be a remarkable exception, as the peridium is set with spines as in *Lycoperdon*. Nothing is known of its microscopical structure (except spores). The only other species considered good by Lloyd is his *A. giganteum*, also from South Africa, and it offers no difficulties so far as the description goes (Myc. Notes, p. 645). A plant from Australia was named by Lloyd *A. rufum* (Myc. Notes, p. 254), but he later doubts its essential distinction from *A. album* (p. 1134). All other so-called species are considered spurious by Lloyd.

Arachnion album Schw.

Plate 49

Plants with the fruiting body above ground, irregularly spherical with a more or less pointed base which extends into a small toughish root, lower surface more or less grooved or pitted, surface smooth, dull white until maturity then buffy when dry, or yellowish if water soaked. Peridium thin, 0.5 mm. thick or less, the same thickness all around, toughish. Gleba pure white then gray or brownish olive at maturity, composed of a large number of minute empty chambers lined by a hymenial layer, composed apparently only of basidia, the context between the chambers composed of a loose weft of delicate, interwoven hyphae. At maturity this loose tissue breaks down and leaves the chambers as distinct granular particles which contain the spores and fall apart like sand or decay into a sordid mass in wet weather. This structure indicates a close affinity to *Lycoperdon*. The peridium has no definite opening but falls to pieces slowly by decay or usually by the attacks of grubs (the larvae of the fire-fly playing a large part). Odor none until ripe, then decided and nitrous, resembling somewhat that of *Bovistella radicata* and some Lycoperdons. There is no capillitium or columella or sterile base.

Spores (of No. 730) brown under the microscope, smooth, short elliptic, with a decided mucro or short stalk about 1μ long, though sometimes twice the length of the spore, 3.2–4.3 $\times$ 4.3–5.5μ. Basidia clavate, 3.7 $\times$ 11μ, apparently bearing only one mature spore at a time though two or four sterigmata may be seen in the young state; sterig-

mata very peculiar, a long, very slender part remaining attached to the basidium and a shorter, stouter part falling off with the spores as its stalk.

It was from this state that Schweinitz first described this interesting plant. It is plentiful in Chapel Hill in summer and seems to prefer cedars, but it also grows in deciduous groves and in open places and we have found it in abundance in flower beds. We have also found it at Hartsville, S. C. It is widely distributed in the eastern United States and, according to Lloyd, in other parts of the world. It has been found only once in Europe (Italy).

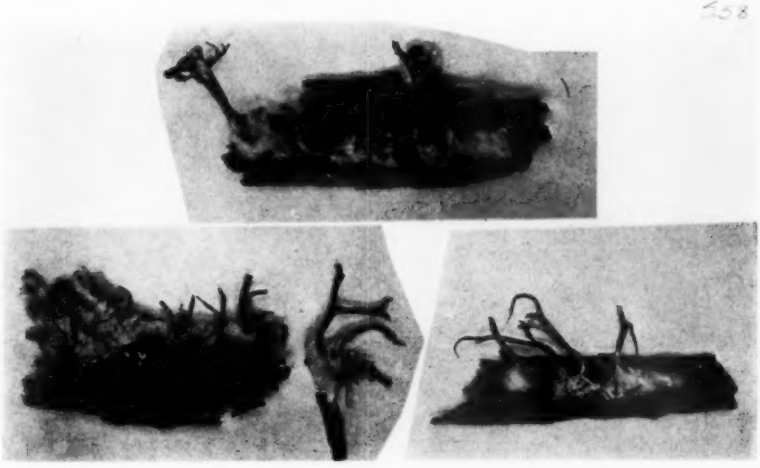
CHAPEL HILL, N. C.

EXPLANATION OF PLATE 49

Fig. 1. Arachnion album. No. 5224 (section of a peridiole).
Fig. 2. Arachnion album. No. 5224.
Fig. 3. Arachnion album. No. 5209.
Fig. 4. Arachnion album. No. 730.
Fig. 5. Geaster saccatus var. Walkeri. Lincoln, Nebraska.
Fig. 6. Boletus parasiticus. Blowing Rock, N. C.
Figs. 7 and 8. Clavaria Stillingeri. Type. Elk River, Idaho.
Figs. 9 and 10. Lachnocladium Micheneri. Ohio. (Lloyd Herb. No. 11.)
Fig. 11. Lachnocladium pusillum. Type. Leyden, Holland.

Fig. 1 × 278; figs. 2 and 3 × 1012; figs. 4–6, 8 and 9 × 1620; figs. 7, 10 and 11 × 810

PLATE 47



[Above] Clavaria Stillingeri. Elk River, Idaho
[Below] Lachnocladium Micheneri. Ohio

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BOLETUS PARASITICUS. BLOWING ROCK, N. C. No. 5686
PARASITIZING SCLERODERMA AURANTIUM

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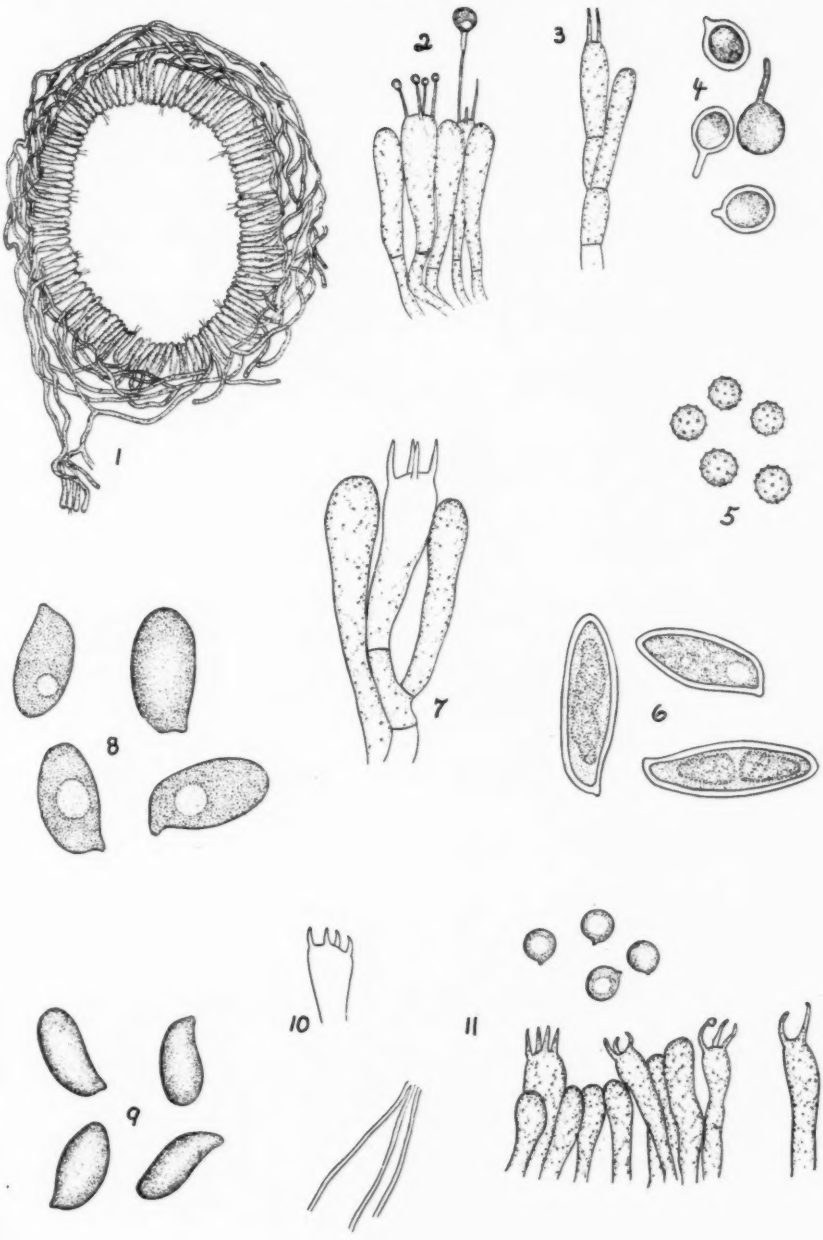
PLATE 49



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